III ASPIC–ASEICA International Meeting
i3S – Porto, Portugal • October 26th & 27th, 2023

Cancer Immunology, Tumor Microenvironment & Metastasis

Proceedings book.

Letter of welcome

Dear Colleagues and Friends,

As Presidents of both ASPIC and ASEICA, it is a pleasure to invite you to participate at the **"III ASPIC-ASEICA International Meeting – Cancer Immunology, Tumor Microenvironment and Metastasis"** that will take place on **October 26th & 27th 2023** at i3S, Porto, Portugal.

We are both affiliated societies of the European Association for Cancer Research (EACR) and, due to common research interests and geographical proximity, have decided to establish a strong link via the organization of joint international scientific meetings and workshops. This meeting is another consolidate step towards that direction. Our main purpose is to disseminate and promote the work developed in both countries and create communication channels and partnership opportunities. We are determined to make the much-needed connections between basic scientists, clinical researchers and oncologists.

We expect to count with the participation of interested scientists in cancer immunology, tumor microenvironment and metastasis from Spain and Portugal, to help us place this event at a high standard level. The meeting will have invited speakers plus talks by attendees selected from abstracts. Participants with posters will also have the opportunity to discuss their work. The posters will be displayed in a specific section in the congress venue.

There will be prizes for the two best Selected Short Talks, which will be awarded by the scientific journals «Journal of Clinical Medicine», «Cancers» and «Immuno» and also EACR prizes for the two best posters. You are invited to take an active part in this meeting, which we believe will be an outstanding scientific event. We are sure that you will enjoy it!

Joana Paredes (ASPIC's President) & **Marisol Soengas** (ASEICA's President)

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Juan Rodríguez Vita (CIPF – Valencia)

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Congress programme

Thursday, 26th October

15:00 Official Opening

Luis Costa (ASPIC's Past President) &
Xosé Bustelo (ASEICA's Past President)

15:15 Opening Lecture - EACR Sponsored Speaker

Chair: **Marisol Soengas** (President of ASEICA)

Understanding site-specific immunity to maintain disseminated tumor cell dormancy

Ana Luísa Correia (Champalimaud Found., Lisboa, Portugal)

16:00 Coffee Break

Session 1. Cancer Immunology

Chair: **Carmen Jerónimo** & **José Carlos Machado**

16:30 The influence of hypoxia and ROS in T cell anti-tumor activity

Asís Palazón (CIC bioGUNE, Bizkaia, Spain)

17:00 Selected Short Talk

Genomic biomarkers for predicting lung cancer immunotherapy response

Ana Oitabén Fernández (CiMUS, Santiago de Compostela, Spain)

17:15 Immunotherapy in brain tumors: Challenges and opportunities

Rui M. Reis (ICVS, Minho University, Braga, Portugal)

17:45 Selected Short Talk

From cold to hot: Nano-immunotherapy driving pancreatic cancer over effective anti-tumor response

Rita C. Acúrcio (iMed.ULisboa/Faculty of Pharmacy, Lisbon, Portugal)

18:00 Patients' Advocacy Lecture

Chair: **Joana Paredes**

EVITA Platform – A citizen-centered support tool driving cancer research

Tamara Hussong Milagre (EVITA)

20:00 Meeting Dinner

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Friday, 27th October

Session 2. Tumor Microenvironment

Chair: **Xosé Bustelo** & **Fátima Baltazar**

9:00 **RAS signalling beyond cancer cells: controlling the physicochemical properties of the extracellular matrix to impact tumour progression**

Esther Castellanos (CIC-IBMCC, Salamanca, Spain)

9:30 `Selected Short Talk`

Impact of HER2+ brain-tropic breast cancer cells in blood-brain barrier dysfunction during the premetastatic niche formation

Liliana Santos (iCBR/CIBB/University of Coimbra, Portugal)

9:45 **The extracellular matrix: a crucial modulator of the tumour immunosuppressive environment**

Maria José Oliveira (i3S, Porto, Portugal)

10:15 `Selected Short Talk`

CTNNA1 germline variants with a premature termination codon are a risk factor for development of early-onset Diffuse Gastric Cancer in European families

Silvana Lobo (i3S/ICBAS, Porto, Portugal)

10:30 Coffee Break

Session 3. Metastasis

Chair: **Célia Gomes** & **Ana Sofia Ribeiro**

11:00 **Novel therapeutic strategies in breast cancer**

Eva González Suárez (CNIO, Madrid, Spain)

11:30 `Selected Short Talk`

UBE2C drives leptomeningeal dissemination in brain metastatic disease and is a promising therapeutic targetn

Eunice Paisana (iMM, Lisboa, Portugal)

11:45 **Systemic metabolic cues affect breast cancer progression**

Sérgio Dias (iMM, FMUL, Lisboa, Portugal)

12:15 `Selected Short Talk`

Nanotechnology-based cancer vaccine to re-educate host immune response against melanoma brain metastases

Bárbara Carreira (iMed.ULisboa/Faculty of Pharmacy, Lisbon, Portugal)

12:30 **Endothelial Notch1 signaling in white adipose tissue promotes cancer cachexia**

Juan Rodríguez Vita (CIPF – Valencia)

13:00 Lunch

14:00 Posters

15:30 Closing Lecture

Chair: **Joana Paredes** (ASPIC President)

Burning metastasis bridges: Targeting metastasis initiating cells in combination with immunotherapy

Hector Peinado (CNIO, Madrid, Spain)

17:00 Awards & Closing Session

Joana Paredes (ASPIC/i3S) &
Marisol Soengas (ASEICA/CNIO)

Abstracts

Opening Lecture – EACR Sponsored Speaker

Understanding site-specific immunity to maintain disseminated tumor cell dormancy

Author and Affiliation

Ana Luísa Correia

Champalimaud Foundation, Lisboa, Portugal

Abstract

Metastatic disease, that is when cancer has spread and presents in distant sites, continues to cause the vast majority of all cancer-related deaths. Despite all disseminating systemically, tumor cells develop asynchronously in different tissues, and may seed metastases within months, years, decades, or not at all within a patient's lifetime. Ana Luisa Correia has a main interest in understanding what makes a tissue favorable or not to metastasis, and in leveraging this biology into therapeutic interventions that reliably prevent the emergence of metastases in patients with cancer. Ana has developed a tool to follow dormant disseminated tumor cells live, offering opportunities to investigate the anatomical distribution, composition and dynamics of dormant reservoirs within and across distant sites. This approach has steered the discovery of a pivotal role for a part of the innate branch of the immune system, the natural killer cells, in lulling disseminated tumor cells into dormancy, and how disruption in liver physiology breaches the NK cell barrier to metastasis. This provides a foundational framework for studying the dynamics of antimetastatic innate immunity within and across sites, which the Correia Lab has been pursuing at the Champalimaud Foundation in Lisbon.

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Curriculum Vitae

Ana Luisa Correia received her B.Sc. in Applied Biology from the University of Minho, Portugal. As a student of the GABBA program, she pursued her PhD studies on the role of the microenvironment in breast cell invasion at the Lawrence Berkeley Lab, USA, and then a postdoc at the FMI and the University of Basel, Switzerland, exploring tissue-specific mechanisms controlling breast cancer progression. Ana is currently a Principal Investigator at the Champalimaud Foundation, where she leads the Cancer Dormancy & Immunity Lab. The goal of her research is to understand how disseminated tumor cells interact with the unique microenvironment in each distant site, and leverage this knowledge into more effective therapeutic interventions for patients risking metastases. Ana has received a few international awards (2021 Metastasis Research Prize, 2022 Pfizer Oncology, AACR 2022 NextGen Stars), is an EMBO Young Investigator, and serves as an active member at the EACR, AACR and MRS societies.

The influence of hypoxia and ROS in T cell anti-tumor activity

Author and Affiliation

Asís Palazón

CIC bioGUNE, Bizkaia, Spain

Abstract

Introduction: Tumour infiltrating lymphocytes (TILs) must exert their anti-tumour activity in the tumour microenvironment (TME), which in turn promotes T cell exhaustion (TEX) limiting the success of cancer immunotherapies. The identification of novel biomarkers of exhausted T cells would offer genuine therapeutic opportunities, broadening the scope of cancer immunotherapy in solid tumours.

Material and Methods: Comprehensive single-cell transcriptomic analyses of tumour-infiltrating T cells from 17 different tumour types (n=77 patients) revealed a strong upregulation of proteasomal genes in exhausted T cells. Multi-colour flow cytometry was used to validate these findings in vitro in human T cells, and in vivo in mouse models. Proteasome modulators (bortezomib, TWS119) were used to explore the pharmacologic potential of this novel biomarker.

Results and Discussions: Single-cell transcriptomics and bioinformatic analyses revealed an enrichment in genes encoding several subunits of the proteasome in exhausted T cells compared to other TILs. TEX is characterized by accumulation of damaged proteins as a result of pathological levels of reactive oxygen species (ROS) and hypoxia in the TME. We generated a proteasome score that strongly correlates with signatures of TEX and ROS in TILs. The ubiquitin-proteasome system is a pathway that maintains proteostasis and cellular fitness through the clearance of ROS-induced damage in the T cell proteome. A variety of assays demonstrated increased proteasome activity in human and mouse exhausted T cells. In this context, pharmacological activation of the proteasome might contribute to alleviate the proteotoxic stress suffered by exhausted T cells and preserve the effector function of TILs.

Conclusion: We found that exhausted T cells in the TME are characterised by increased expression and activity of the proteasome. Modulation of proteasomal activity is a novel strategy to prevent exhausted T cell dysfunction promoted by ROS and hypoxia in the TME of solid tumours.

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Curriculum Vitae

I joined CICbiogune in 2019, where I lead the Cancer Immunology and Immunotherapy lab supported by an ERC Starting grant. Before, I was a senior scientist at AstraZeneca (Cambridge, UK). The aims of our lab have a translational and innovation-oriented perspective, and are focused on the development of immunotherapies for the treatment of solid tumors. Particularly, we are interested in the influence of specific features of the TME (i.e., hypoxia) on T cell responses. In 2022, I founded a spin-off company (Zelula biopharma) with a focus on targeting the TME of solid tumors as a differential approach to cancer immunotherapy.

Selected Short Talk

Genomic biomarkers for predicting lung cancer immunotherapy response

Authors and Affiliations

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3. Liquid Biopsy Unit. Translational Medical Oncology Group. IDIS. Complexo Hospitalario Universitario de Santiago de Compostela, Santiago de Compostela, Spain.

Abstract

Lung cancer (LC) represents the leading cause of cancer-related death worldwide. Although immune checkpoint inhibitors (ICI) show promising results, the majority of patients do not benefit from it, underscoring an urgent need for reliable predictive biomarkers. We examined the possibility to obtain a reliable predictive signature by performing a comprehensive genomic analysis through the evaluation of a) single nucleotide variants (SNV), b) Tumoral Mutational Burden (TMB), c) Copy Number Alterations (CNA) and d) Line-1 retrotransposon activation (L1-RT). We analysed samples from advanced LC patients treated with ICIs at first line following two approaches: (1) liquid biopsy samples from 25 patients to evaluate SNVs, TMB and CNAs using QIAseq Target DNA Lung Panel (Qiagen); (2) FFPE tumor samples from 31 patients to undergo our novel method called RetroTest, designed to evaluate L1-RT based on L1 integrations. We identified SNVs in important cancer-related genes associated with 3-month radiological response. We obtained a promising and non-invasive mutational signature composed of only 10 genes, which showed a high predictive capacity for ICI response (AUC = 0.98). Additionally, our analysis revealed a trend where responders (R) seemed to exhibit more CNA compared to non-responders (NR), although not reaching statistical significance. We did not observed differences in blood TMB. Interestingly, RetroTest results revealed that R in the radiological evaluation tend to have high L1-RT genomic activation (80%).

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These findings highlight the potential of assessing diverse genomic features for predicting ICI response in LC that could be combined to create a robust biomarker signature. Although a larger cohort needs to be evaluated to validate the potential of CNA and L1-RT activation, the 10-gene predictive signature together with SNV in crucial cancer genes clearly demonstrate a predictive power measured in liquid biopsies.

Immunotherapy in brain tumors: Challenges and opportunities

Author and Affiliation

Rui M. Reis

ICVS, Minho University, Braga, Portugal

Abstract

Brain tumors, both malignant and benign, pose a significant medical challenge with limited treatment options and a dismal prognosis for many patients. Traditional therapies, such as surgery, radiation, and chemotherapy, have shown limited success in managing malignant brain tumors. In recent years, immunotherapy has emerged as a promising and innovative approach in the fight against cancer. However, the efficacy of immunotherapy in brain tumors, particularly glioblastomas, and medulloblastomas, some of the most frequent and aggressive subtypes, is limited.

The present seminar will address the immune expression profile of both GBM and medulloblastoma, the identification of prognostic and potential novel targets of immune approaches in these tumors, and the development of models to explore these putative therapies.

Curriculum Vitae

Scientific Director of the Barretos Cancer Hospital, Barretos, SP. Brazil; Invited Associate Professor at the School of Medicine, University of Minho (Braga, Portugal); and Extraordinary Professor, Pan African Cancer Research Institute, University of Pretoria (South Africa).

Degree in Biology at University of Porto, Portugal; PhD in Neuro-Oncology at the IARC, Lyon, France / University of Porto, Portugal; and post-doc in Cancer Genomics at VUMC -Free University Medical Center, Amsterdam, Netherlands / IPATIMUP, Porto, Portugal. Involved in Cancer Research for over 25 years, author of more than 350 articles focusing on molecular pathology and cancer biomarkers of distinct solid tumors.

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From cold to hot: Nano-immunotherapy driving pancreatic cancer over effective anti-tumor response

Authors and Affiliations

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3. Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

Abstract

Immune checkpoint inhibitors (ICI) revolutionized cancer treatment. However, clinical trials have shown PDAC as one of the most ICI-resistant cancers. In fact, PDAC has proven to be refractory to the most conventional and modern anti-neoplastic therapies. Looking into PDAC, the unique dense stroma, and the highly immunosuppressive tumor microenvironment (TME) have limited the infiltration by immune cells and therapeutics. Here, we show the immune cell recruitment into the PDAC microenvironment using a synergic combination of a nanotechnology-based platform and FAK inhibition.

We prepared poly(lactic acid) (PLA) and poly(lactic-co-glycol) (PLGA)-based nanoparticles (NP) co-entrapping PDAC-associated antigens, selected using an immune-bioinformatic analysis, and immune regulators following an established double emulsion method. NP were characterized by dynamic light scattering and atomic force microscopy (AFM). The anti-tumor effect was evaluated in the PDAC KPC-bearing mouse model. The immune profiling of the spleen and TME cells was performed by spectral flow cytometry.

Stable and safe NP were obtained. AFM showed spherical NP with a slightly rough surface, having high entrapment efficiency (70-90%). Cy5-labeled NP were extensively internalized by dendritic cells triggering their activation (higher levels of CD80, CD86, and CD40 co-stimulatory molecules). NP and FAK inhibitor (FAKi) successfully recruited cytotoxic T cells into the TME, controlled cancer-associated fibroblast phenotypes, and restrained tumor growth.

The developed NP induced an effective antigen-specific immune response against PDAC. In addition, the combination of NP and FAKi, unlocked the dense and immunosuppressive PDAC microenvironment to immune cell infiltration.

EVITA Platform – A citizen-centered support tool driving cancer research

Author and Affiliation

Tamara Hussong Milagre

EVITA

Abstract

Along 12 years of activity, Patient Association EVITA has identified the most burning unmet needs in hereditary cancer research and development. During the last 5 years EVITA developed a digital platform to close the gaps. EVITA Platform not only offers digital support and risk management to citizens and patients, but it has also a strong focus on patient generated data to boost research through innovative RWD and RWE.

Curriculum Vitae

Tamara Hussong Milagre is an accomplished leader and visionary in the field of hereditary cancer advocacy. With an unwavering commitment to improving the lives of families affected by Hereditary Cancer Syndromes, she has left an indelible mark since founding the pioneering patient association, EVITA – Cancro Hereditário (=AVOID Hereditary Cancer), in 2011. At the European level, she serves as a member of the ePAG Council at the prestigious European Reference Network GENTURIS, dedicated to Genetic Tumor Risk Syndromes. Tamara is deeply engaged in thematic areas such as "Hereditary Breast and Ovarian Cancer (HBOC)" and critical taskforces on "Organization of Care" and "Education, Training and Development."

Tamara took part in the Master Class of the European School of Oncology ESO, tailored for leading patient advocates. Her evolution into a Patient Expert, achieved through the rigorous EUPATI course, underscores her profound understanding of the pivotal role patients play in shaping the research and development of medicines. Later, she graduated from the EURORDIS Leadership School on Healthcare & Research in 2019.

Tamara's dedication and outstanding contributions to the field of hereditary cancer advocacy were officially recognized in November 2020 when she was honored with the prestigious Medal of Scientific Merit by the Portuguese Minister of Science.

RAS signalling beyond cancer cells: controlling the physicochemical properties of the extracellular matrix to impact tumour progression

Authors and Affiliations

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4. Cancer Evolution and Genome Instability Laboratory, The Francis Crick Institute, London NW1 1AT, UK

Abstract

Lung cancer remains a clinical unmet need and a significant cause of death. KRAS is mutated in 30% of non-small cell lung cancer (NSCLC), the most frequent type of lung cancer. Recently, the FDA approved the first RAS-targeted therapies for NSCLC with RASG12C mutations, but patients harboring other mutations are not eligible for treatment. Within the tumor microenvironment, cancer-associated fibroblasts (CAFs) play a crucial role in shaping tumor behavior. Among these, two distinct subtypes, inflammatory CAFs (iCAFs) and myofibroblastic CAFs (myoCAFs), exhibit differing functions. iCAFs are characterized by their involvement in immune response modulation, secreting pro-inflammatory cytokines, and contributing to an immunosuppressive environment that aids tumor immune evasion. On the other hand, myoCAFs possess contractile properties and contribute to tissue remodeling, angiogenesis, and extracellular matrix deposition, thereby facilitating tumor growth and metastasis. These differing functions of iCAFs and myoCAFs contribute to heterogeneous tumor behavior, influencing factors such as tumor invasion, angiogenesis, and immune cell infiltration. The distinct phenotypes of CAFs can thus lead to varying therapeutic responses and clinical outcomes, highlighting the importance of understanding their roles in the context of tumor biology. Here we show

that in NSCLC models, CAFs rely on RAS-PI3K signaling for normal function and acquisition of an iCAF or myoCAF phenotype. Furthermore, genetic disruption of RAS-PI3K interaction, or pharmacological inhibition of PI3K p110α activity with BYL719 in murine and human CAFs, results in a shift towards an iCAF phenotype characterized by heightened IL6 production and diminished α-smooth muscle actin (α-SMA) levels.As a consequence, CAFs defective in RAS-PI3K form thinner and more disorganized extracellular matrices that are defective in components such as collagen or fibronectin, profoundly impacting macrophage and lymphocyte function. These defective ECMs also compromise proliferation, activation of epithelial to mesenchymal programs (EMT) and migration potential of several KRAS mutant lung cancer cell lines. Additionally, experiments in mice with fibroblast-specific disruption of RASPI3K interaction (Pik3caRBD/Lox/Col1a2CreER/WT) show that disrupting this interaction in CAFs causes a significant delay in KRAS-driven lung tumor growth and changes in CAF activation, ECM composition and immune modulation, further demonstrating the key role of RAS-PI3K interaction in CAFs for cancer progression. In summary, this study highlights the potential of targeting the tumor microenvironment, specifically cancer-associated fibroblasts (CAFs), as a novel mutation-agnostic approach. The identification of RAS-PI3K signaling's pivotal role in CAF phenotype acquisition further emphasizes its significance in orchestrating tumor progression. These findings provide valuable insights for developing innovative therapeutic strategies to address the complexity of NSCLC, addressing the urgent need for more effective treatment options

Curriculum Vitae

Esther Castellano leads the Tumor:Stroma signalling group at Centro de Investigación del Cáncer in Salamanca. Her lab focuses on understanding how oncogenic RAS proteins drive lung cancer development by regulating the interplay between tumour cells and its microenvironment. She completed her PhD in Eugenio's Santos Lab at Centro de Investigación del Cáncer in Salamanca, where she uncovered that HRAS and NRAS oncogenes have specific functions. After finishing her PhD Esther moved to Julian Downward's Lab at London Research Institute, now part of the Francis Crick Institute, in London where she continued working on RAS oncogenes, but this time on the role of RAS activation of PI3K in lung cancer. After her postdoctoral training she moved to Barts Cancer Institute as an Early Career PI and in 2018 she moved to Centro de Investigación del Cancer in Salamanca as an independent researcher

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Impact of HER2+ brain-tropic breast cancer cells in blood-brain barrier dysfunction during the premetastatic niche formation

Authors and Affiliations

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5. I3S - Institute for Research and Innovation in Health, University of Porto, Portugal.

Abstract

Introduction: Up to 50% of HER2+ breast cancer patients eventually develop brain metastasis (BM), with a median survival of less than 1 year after diagnosis. Disruption of the blood-brain barrier (BBB) is a crucial step for metastatic cells to enter the brain. However, the pathways that drive these events remain poorly understood. Here, we evaluated how metastatic breast cancer overcomes the BBB for extravasation and brain colonization.

Material and method: An in vitro BBB model was exposed to the secretome derived from HER2+ BCCs and their brain-tropic variants. BBB integrity was assessed by measuring the transendothelial flux of a 4kDa-dextran, the TEER and the expression of tight and adherens junction proteins. Foxn1nu/nu mice were: i) pretreated with BCC-derived secretome, ii) injected orthotopically with BCCs into the mammary fat pad for primary tumor formation; iii) injected intracardially with BCCs for BM formation. BBB integrity was assessed in vivo by near-infrared fluorescence imaging, and ex vivo by collagen IV and albumin immunostaining in the brain. Primary tumor and BMs formation were monitored by bioluminescence imaging.

Results: Brain-tropic cells secrete specific bioactive factors that disrupt the BBB both in vitro and in vivo. Animals pretreated with brain-tropic cell secretome showed structural changes in the BBB, as evidenced by a decrease in collagen IV and an increase in albumin immunoreactivity, along with the

accumulation of 20kDa dextran into the brain. These structural alterations were also observed in animals harboring a localized primary tumor without BM, suggesting a systemically mediated effect. Importantly, these changes in BBB permeability facilitated the formation of BM.

Conclusion: Our findings underscore the selective disruption of the BBB by secreted factors from brain-tropic cells, allowing cell extravasation and subsequent BM formation. Restoration of proper endothelial function should be exploited to prevent BMs.

The extracellular matrix: a crucial modulator of the tumour immunosuppressive environment

Author and Affiliation

Maria José Oliveira

i3S, Porto, Portugal

Abstract

The extracellular matrix is one of the most abundant elements of the tumor microenvironment. It is dynamically synthesized and remodeled by cancer and stromal cells, while modulating cancer progression and response to therapy. In colon cancer, high stromal content is frequently associated to poor patient prognosis, but the underlined mechanisms remain to be elucidated. Therefore, we established the biomechanical and biochemical signature of paired normal and tumor decellularized matrices, derived from colon cancer patients surgical resections. This detailed characterization unraveled that human tumor matrices are stiffer, comprising a denser fiber network, and arresting secreted factors associated to TGF-beta signaling and fibroblasts activity. Interestingly, left-sided tumors, generally less infiltrated by immune cells and less responsive to immunotherapy, are stiffer than right-sided tumors, exhibiting a distinct molecular signature.

Importantly, we also demonstrated that decellularized tumor matrices, in contrast to their normal counterparts, promoted cancer cell stemness and skewed macrophages towards an anti-inflammatory phenotype with the ability to express CCL18, an immunosuppressive pro-fibrotic and pro-invasive chemokine. The mechanisms underlying CCL18-mediated cancer cell invasion are currently being dissected and the putative receptor investigated. In addition, assessing a CRC clinical cohort, we revealed that CCL18 is highly expressed at the invasive front of human CRC tumors, correlating with advanced tumor staging. Additional results from clinical public databases also evidenced CCL18 association with worse overall survival and disease-free survival, particularly in patients with MMR deficient tumors, generally with higher immune cell infiltration. This study established the biomechanical and biochemical signature of CRC patients matrices and highlight its ability to dictate immune cells function and the creation of an immunosuppressive environment.

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Curriculum Vitae

Maria J Oliveira is the Coordinator of the Tumour and Microenvironment Interactions (TMI) Group and Prinicipal Researcher at i3S- Institute for Research and Innovation in Health, at the University of Porto (Portugal). In 2004, she received her PhD in Health and Medical Sciences at the University of Ghent (Belgium), studying the role of bacteria on colorectal cancer invasion and dissecting the associated mechanisms. After a PostDoc at IPATIMUP (UPorto) working on gastric cancer invasion-associated signalling pathways, Maria joined the Institute for Biomedical Engeneering (INEB, UPorto), where she initiated a new line of research. In 2017, Maria established the TMI group, focused on studying the role of the tumor microenvironment, particularly of immune cells, adipocytes and extracellular matrix components, on the modulation of cancer cell invasion and metastasis. Maria's research is also dedicated to understand how cancer cells escape immune surveillance, foreseeing the design of more efficient immunomodulatory therapies. Along her career she has co-authored over 110 publications and has received several awards as the Yamagiwa-Yoshida Award and the Medals L'Óreal Women in Science.

CTNNA1 germline variants with a premature termination codon are a risk factor for development of early-onset Diffuse Gastric Cancer in European families

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Abstract

Introduction: Rare CTNNA1 variants occur in Hereditary Diffuse Gastric Cancer and Macular Dystrophy Patterned 2 patients. We aim to understand if CTNNA1 variants cause specific phenotypes depending on variant gene location, molecular type and/or geography.

Methods: We developed a worldwide CTNNA1 variant clinical database, curated for population frequency, geographic origin, molecular impact and clinicopathological features. In silico tools predicting premature termination codons (PTC) categorized variants as PTC or non-PTC; multivariable logistic regression analyzed genotype-phenotype associations; NMD degradation assays tested nonsense mediated mRNA decay (NMD) blockade, and; a humanized Drosophila model was built to test variants' functional impact.

Results: 172 CTNNA1 variants (FREQ<0.1%) were found in 344 families (1802 phenotypes; 1604 individuals), 76% predicted as PTC. Eye disorders were significantly associated with non-PTC families (OR=195.66; p<0.001) in the worldwide cohort. Only in Europe, diffuse gastric cancer (DGC: μ age of onset=47.4±13.9yo) most likely occurs in PTC than non-PTC families (OR=13.87; p<0.001), while breast cancer of unknown histotype most likely occurs in non-PTC families (OR=2.333; p=0.035). NMD blocking promoted 13-fold mRNA increase of CTNNA1 PTC-bearing transcripts. In αE-catenin knock-out Drosophila, organ development/lethality was rescued by a human αE-catenin non-PTC protein, but not by PTC-bearing αE-catenin.

Conclusion: In European families, early-onset DGC was independently associated with CTNNA1 PTC variants, which we proved to be targeted for NMD. The Drosophila humanized model we developed is suitable to assess functional impacts of germline CTNNA1 variants from cancer families. CTNNA1-mutant tumors' ongoing somatic analysis is expected to highlight variant-specific dysregulated mechanisms.

Novel therapeutic strategies in breast cancer

Author and Affiliation

Eva González Suárez

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Abstract

RANK signaling pathway has emerged a therapeutic target for breast cancer prevention and treatment. I will discuss previous data showing how RANKL inhibition prevents or attenuates BC initiation, reduces recurrence and metastasis and induces tumor cell differentiation in established tumors. Moreover, RANK signaling also modulates the tumor microenvironment. Given BC heterogeneity, the identification of breast cancer patients who may benefit from denosumab remains a challenge. Ongoing efforts to identify these patients will be discussed.

Curriculum Vitae

Dra. Eva Gonzalez is PhD in Molecular Biology, is a Senior scientist and group leader of the Transformation and Metastasis laboratory, at the Spanish National Cancer Research Center (CNIO) in Madrid, Spain.

Her academic merits and research have been recognized with an ERC-Consolidator grant, ERC-Proof of Concept, a Susan Komen career catalyst grant, and several awards. She is member of experts committee in several institutions as the Agencia Estatal de Investigación, European network of breast development and cancer (ENBDC), among others, and has supervised several PhD students and postdocs.

Research in her laboratory aims to identify novel targets for breast cancer treatment and understand mechanisms of resistance to current drugs. She has more than 50 publications, half of them in D1 and half as corresponding author.

To highlight a topic of her extensive research career, one of her main contributions of research has focused on the role of RANK in mammary epithelial homeostasis, breast cancer and tumor immunology, with studies from basic to clinical research, including the coordination of a clinical trial in breast cancer patients.

UBE2C drives leptomeningeal dissemination in brain metastatic disease and is a promising therapeutic target

Authors and Affiliations

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Abstract

Brain metastases (BM) are a frequent late-stage complication in cancer patients and one of the main causes of death. Current treatment options often fail mainly due to the poor crossing of systemic therapies through the blood-brain barrier and the genetic differences that exist between the primary tumor and brain lesions. We performed RNA sequencing in thirty BM from patients with different primary tumor origins. We found that UBE2C, a gene involved in the correct transition from metaphase to anaphase, is upregulated in patients with BM. Using an independent cohort of patients, we demonstrated that high UBE2C expression was associated with decreased survival. Cancer cells with overexpression of UBE2C have increased migration and invasion capacities in vitro. Moreover, overexpression of this gene was able to decrease mouse survival in orthotopic xenografts of BM, and induced leptomeningeal dissemination, an aggressive metastatic phenotype. Treatment with a PI3K/mTOR inhibitor effectively prevented this phenotype, blocking cancer cell signaling and decreasing UBE2C levels. These data point out UBE2C as a molecular marker of poor prognosis in cancer patients with BM and a relevant player in brain metastatic disease. Our results also validated a PI3K/mTOR inhibitor as an effective therapy against UBE2C-driven brain metastatic disease.

Systemic metabolic cues affect breast cancer progression

Author and Affiliation

Sérgio Dias

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Abstract

Most metastasis from breast cancer occur via the dissemination of tumor cells through the bloodstream. How tumor cells enter the blood (intravasation) is, however, a poorly understood mechanism at the cellular and molecular levels. Particularly uncharacterized is how intravasation is affected by systemic nutrients. High levels of systemic LDL-cholesterol have been shown to contribute to breast cancer progression and metastasis in various models, but the cellular and molecular mechanisms involved are still undisclosed. We have exploited the metabolic, biochemical and cell biology basis for the effects of elevated systemic cholesterol. We have shown that LDL cholesterol affects mitochondrial dynamics and function, and affects lipid uptake and processing at mitochondria, resulting in accumulated ROS levels and increased migratory/invasive behavior by breast cancer cells. We have further shown that LDL promotes vascular invasion in vitro and favors the intercalation of tumor cells with endothelial cells, a phenotypic change resembling vascular mimicry (VM). At the molecular level, LDL increases the expression of SERPINE2, previously shown to be required for both VM and intravasation.

Overall, our studies are unraveling novel mechanisms by which systemic hypercholesterolemia may affect the onset of metastatic breast cancer by favouring metabolic and phenotypic changes in breast cancer cells and increasing intravasation.

Curriculum Vitae

Sérgio Dias obtained his degree in Biology at the Faculty of Sciences of the University of Lisbon, completed in 1994. He then took his PhD in Tumor Immunology (focusing on animal models of breast cancer) in London, at the Imperial Cancer Research Fund (1995-1998).

Sérgio Dias carried out his post-doctoral training at Cornell University, in New York, in the USA between 1999 and 2001. After this period he returned to Portugal and established his laboratory at the Portuguese Institute of Oncology, in Lisbon. He was Assistant Researcher and Director of the Department of Molecular Pathobiology at the IPO of Lisbon for 10 years, during which he was also a Guest Assistant Professor at the Faculty of Medical Sciences of the Nova University in Lisbon (2009-2012).

Currently, Sérgio Dias is Principal Investigator at the João Lobo Antunes Institute of Molecular Medicine and Guest Associate Professor at the Faculty of Medicine of the University of Lisbon (since 2013). He is also Co-director of the IMM Biobank since 2014 and Coordinator of the National Biobank Network (which is part of the FCT National Infrastructure Network).

To date, he has supervised 14 PhD students, 25 Master's students and 12 post-doctoral researchers. The research carried out in his laboratory, focused on the study of cancer as a systemic disease (namely on the mechanisms of metastasis formation), resulted in the publication of 82 articles in indexed international journals, with 16,000 citations and an h factor of 44 (September 2023).

Sérgio Dias has been teaching cancer biology to undergraduate students and the general public for over 15 years, having given lectures on this topic at several venues, such as Schools and Institutes in the Greater Lisbon area. He also writes a monthly article in Visão, a general-knowledge based magazine, popular in Portugal.

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Nanotechnology-based cancer vaccine to re-educate host immune response against melanoma brain metastases

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Abstract

Despite the remarkable efficiency of immune checkpoint inhibitors (ICI) against metastatic melanoma, only a small percentage of patients respond to these therapies. ICI inefficiency is being attributed to tumor-related immunosuppression and limited infiltration of T cells. To overcome these limitations, new strategies are needed. Here, we report the development of a cancer nanovaccine (NV) to specifically target dendritic cells (DC) and modulate melanoma-immune cell interactions.

We designed and characterized poly(lactic acid) (PLA)-based NV using mannose-grafted polymers to deliver combinations of melanoma neoantigen, toll-like receptor ligands, and regulators of the PD-1/PD-L1 pathway. These cancer NV amplified antitumor immune responses by increasing antigen processing, and presentation to effector T cells. NV were shown to have spherical shape (180 nm), narrow polydispersity index, near-neutral surface charge, and high loadings of the immune regulators. Both subcutaneous and intranasal immunization induced the activation and maturation of DC within draining lymph nodes and triggered the systemic activation of neoantigen-specific cytotoxic T cells. Treatment with the combination of the NV with PD-1/PD-L1 modulators in vivo led to increased tumor inhibition in primary melanoma model. Combination of the NV with the PD-L1 antibody was the most effective, with maximal tumor inhibition, translated into high cytotoxic CD8+ T cells infiltration into the tumor microenvironment and reduced expression of immunosuppressor cells. At the metastatic disease, the intranasal immunization of the NV combined with the PD-L1 ICI led to the prevention of melanoma brain metastases. Combination strategy led to 100% survival at 52 days followed by tumor inoculation, and recapitulation into a T-cell inflamed brain microenvironment.

Altogether, the synergy between the NV and the PD-L1 antibody provides essential insights to devise alternative combination regimens to improve the efficacy of ICI in metastatic melanoma.

Endothelial Notch1 signaling in white adipose tissue promotes cancer cachexia

Author and Affiliation

Juan Rodríguez Vita

CIPF – Valencia

Abstract

Cachexia is a major cause of morbidity and mortality in cancer patients characterized by weight loss, adipose and muscle tissue wasting. Hallmarks of white adipose tissue (WAT) remodeling, which often precedes weight loss in patients and mice, are impaired lipid storage, inflammation, and eventually fibrosis. Tissue wasting occurs in response to cancer-secreted factors, however, the continuous endothelium in WAT is the first line of contact with circulating factors and actively regulates tissue accessibility through transendothelial movement. This raises the question whether the endothelium itself may orchestrate tissue remodeling. Endothelial cells comprise the inner layer of blood vessels forming a highly dynamic barrier controlling the active transport of metabolites. Despite the enormity of data that is focused on the impact of dysregulated adipocyte function to the development of metabolic diseases, little is known on the contribution of the adipose tissue endothelium to disease progression. We found that, in the pre-cachexia phase, tumors excessively activate the Notch1 signaling pathway in distant WAT endothelial cells, from human and mouse cancer models. Our group has previously identified the endothelial Notch1 signaling pathway as a key contributor to cancer metastasis. Through the use of mouse models, human adipose tissue biopsies and organoid cultures, we were able to show that the endothelial Notch1 signaling pathway regulates adipose tissue vitamin A metabolism in both endothelial cells and recruited macrophages. Vitamin A is a fat-soluble metabolite that is primarily stored in the liver and adipose tissue in its inactive form. Beneficial and therapeutic effects that have been shown to occur upon injection of retinoic acid (RA), the active form of vitamin A. Mechanistically, endothelial Notch1 signaling increased RA and IL33 synthesis in ECs. The angiocrine factor IL-33 induced excessive RA production in neighboring adipocytes and macrophage, exerting a continuous resolution of inflammation in WAT. Targeting RA signaling, downstream of Notch, using an oral pan-RAR antagonist inhibited WAT wasting in the pancreatic carcinoma KPC model without inducing the Notch-dependent side effects. Taken together, the adipose tissue endothelium is capable of mediating WAT wasting in an angiocrine manner, where RA signaling serves as a newly identified targetable pathway.

Curriculum Vitae

Dr. Juan Rodríguez Vita obtained his PhD at the FJD in Madrid (Spain), and after postdoctoral periods at the CIML in Marseille (France), the IDIBAPS in Barcelona (Barcelona) and the DKFZ in Heidelberg (Germany), where he also assumed a project leader position, he started his own group at the CIPF in Valencia (Spain) on September 2021. His research focuses on deciphering the communication signals between tumor cells and stroma. Juan Rodriguez-Vita on PubMed.

Closing Lecture

Burning metastasis bridges: Targeting metastasis initiating cells in combination with immunotherapy

Author and Affiliation

Hector Peinado

CNIO, Madrid, Spain

Abstract

The development of treatments targeting PD-1/PD-L1 axis has revolutionized the field of cancer immunotherapy (IT). In advanced melanoma, these therapies have significantly improved patient outcomes (1, 2). However, not all patients respond to IT, and those who do usually develop therapy resistance (3). **Therefore, there is a need for novel therapeutic strategies to overcome IT resistance or enhance the effectiveness of IT.**

NGFR is a critical molecule for melanoma metastatic cells, and its expression is correlated with PD-L1 expression, the suppression of cytotoxic T lymphocytes (4), and immune cell exclusion (5). **We hypothesize that therapies targeting NGFR could potentially rekindle anti-tumor responses against metastatic cells.** We have analyzed NGFR expression in primary tumors from melanoma patients undergoing IT. Our findings revealed an inverse correlation between NGFR expression and progression-free survival in melanoma patients receiving IT. Over the past few years, we have investigated the impact of the NGFR small molecule inhibitor THX-B. Our data support the idea that inhibiting NGFR using THX-B can reduce metastasis in melanoma models. Furthermore, our analysis of the combined treatment of THX-B with anti-PD-L1 showed a decrease in metastasis, **suggesting that combining THX-B with immunotherapy may be an effective approach to combat melanoma metastasis.**

In summary, our data support the idea that **therapeutic intervention against NGFR, in combination with anti-PD-L1, holds promise for reactivating anti-tumor immune cell responses in metastatic melanoma.** Our long-term objective is to develop novel therapies that can reactivate anti-tumor responses against metastatic cells.

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Curriculum Vitae

I did my PhD in the laboratory of Dr. Amparo Cano in Madrid (Spain, Instituto de Investigaciones Biomédicas "Alberto Sols") where I specialized in the analysis of Mechanisms involved in the Epithelial to Mesenchymal Transition. I joined Dr. Lyden's lab at Weill Cornell Medical College as a postdoc in 2008 to study the mechanisms of communication between tumor cells and bone marrow-derived cells during metastatic progression. My work defined that tumor-secreted exosomes educate hematopoietic progenitors toward a pro-angiogenic and pro-metastatic phenotype. Since 2015 I am the Group Leader of the Laboratory of Microenvironment and Metastasis at CNIO. My laboratory is investigating the role of tumor-secreted extracellular vesicles in pre-metastatic niche formation, their potential applications in liquid biopsies and the influence of the microenvironment in metastatic dissemination. My contributions to the field have been recognized with the 1st ASEICA Young Investigator, Pfizer, and "Doctores Diz Pintado" awards. I have been honored as a FERO, Marie Curie-WHRI-Academy and BBVA Leonardo Fellow. I have published 106 works since 2003 and listed in 2022 among the top 2% of most cited scientists worldwide, according to the Stanford University ranking based on Scopus.

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01. Uncovering BMP6 and CXCL12 as deregulated cytokines in bladder tumor microenvironment controlled by epigenetic mechanisms

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Abstract

Introduction: Epithelial-mesenchymal transition (EMT) and tumor microenvironment (TME) play a crucial role in bladder cancer (BlCa). Epigenetic reprogramming has been associated with TME modulation, including cytokine regulation. We aim to understand the interplay between TME and EMT in BlCa and to uncover epigenetic mechanisms behind cytokines regulation.

Materials and Methods: RT2 Profiler PCR Array Human Cytokines/Chemokines was performed using 4 non-muscle invasive BlCa (NMIBCs), 5 muscle-invasive BlCa (MIBCs) and 5 normal tissues. CXCL12 and BMP6 were evaluated in TCGA database and in IPO-Porto BlCa cohort.

Normal bladder cell line SV-HUC1 and BlCa cell lines, RT-112, 5637, J82, UMUC3 and 253J, were treated with recombinant CXCL12 and BMP6 and classical EMT-related genes were analyzed. Furthermore, BlCa cell lines were treated with decitabine (DAC), trichostatin A (TSA) and combination, and cytokine expression was evaluated.

Results: CXCL12 transcript levels were significantly lower in MIBCs than in normal samples, while CXCL12 were significantly increased in MIBCs comparing with NMIBCs patient samples. BMP6 was significantly downregulated in BlCa patient tissues, comparing with normal tissues. CXCL12 treatment regulated the expression of mesenchymal-related genes, with SV-HUC1 and J82 showing a significant increase of CDH2 and ZEB2 and a significant reduction of MMP1. Overall, BMP6 treatment seems to activate the expression of mesenchymal-related genes, since UMUC3 and 253J showed increased CDH2, SNAI1, SNAI2 and ZEB2 levels. Moreover, our results indicate that CXCL12 and BMP6 are epigenetically regulated, mainly by histone acetylation, as transcript expression was upregulated in TSA-treated cell lines. Moreover, 5637 DAC-treated cells showed BMP6 level upregulation, also implicating DNA promoter methylation in gene expression regulation.

Conclusion: We unraveled CXCL12 and BMP6 as crucial cytokines deregulated in BlCa TME, putatively regulated by epigenetic mechanisms.

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02. Potential role of Hakai in N6-methyladenosine (m6A) RNA modification in colon cancer cells

Authors and Affiliations

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Abstract

Introduction: mRNA methylation is one of the most common internal RNA modifications in eukaryotes and has emerged as a widespread regulatory mechanism that controls gene expression in various physiological and pathological processes. m6A is installed by m6A methyltransferases ("writers"), removed by demethylases ("erasers"), and recognized by m6A-binding proteins ("readers"). Recently, it was reported that Hakai, an E3 ubiquitin-ligase for E-cadherin, interacts with m6A writer components. However, up to now, the role of Hakai in m6A mRNA methylation in mammals is not fully understood. In this work, we aim to determine the possible role of Hakai in colon cancer cells through its regulation in m6A.

Materials and methods: RNA immunoprecipitation (RIP) of m6A has been performed in Hakai silencing HT29 cells compared to control HT29 cells, by using an inducible lentivirus system. RNA samples are extracted with trizol and reverse transcription was subsequently performed with the SuperScript VILO cDNA Synthesis kit. A cDNA library was constructed and the human gene expression profile of the transcriptome was analysed by Ion AmpliSeq™ Ion and Torrent 5S/XL sequencer. Bioinformatic data analysis was carried out to elucidate the differentially expressed genes. Differentially expressed genes of interest were validated by Q-PCR.

Results: our work helped us to add functional and molecular insights into the mechanism of Hakai and its implication in m6A regulation in colon cancer cells. Specifically, Hakai has been related to the regulation of the immunological microenvironment in colorectal cancer, where it regulates the methylation of multiple cytokines.

Conclusions: in this work, we found out that Hakai is involved in m6A in colon cancer cells and could play an important role in microenvironment by its influence on several important cytokines.

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03. Rectal cancer radioresistance: Iron metabolism and immune microenvironment as key players?

Authors and Affiliations

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Abstract

Rectal carcinoma is a major player in gastrointestinal tract malignancies high incidence and mortality rates, accounting for 40% of the worldwide colorectal cancer burden.Neo-adjuvant (chemo)radiotherapy is the standard treatment for this type of cancer, once 50% of all cases present an advanced disease at diagnosis. However, more than 30% of patients become resistant to therapy. As such, there is an urgent need to develop more efficient therapies to be combined with radiotherapy. One of the strategies for overcoming treatment resistance can be focused on dissecting radioresistance mechanisms within the iron metabolism and immune compartment at the tumour microenvironment. To tackle this premise, we developed a 3D rectal cancer immune-spheroid model composed of rectal cancer cells, macrophages, and T lymphocytes. Following this, immune-spheroids were subjected to ionizing radiation, mimicking the short-scheme treatment of rectal cancer patients (5 gray/5 days), being further characterized and submitted to high-resolution mass spectrometry for proteomic analysis, nanostring transcriptomics as well as metallomics for immune response analysis and metal quantification.

So far, our findings suggest that radiotherapy induces proliferation, enhances the metabolic activity of irradiated immune spheroids, and affects immune population viability selectively, with macrophages

being more radioresistant than T lymphocytes. Importantly, we observed that macrophages and T lymphocytes potentially protect cancer cells from ionizing radiation. Moreover, the expression of metal (e.g. NGAL, HEPHL1, FPN1), metabolic transporter proteins (e.g. SLC1A3), and immune cell inhibitors (e.g. CD276) were found to be changed in irradiated immune spheroids.

Future work regarding the validation of the novel rectal cancer radioresistance signatures in a rectal cancer cohort will be performed, highlighting their clinical relevance as possible targets to decrease rectal cancer resistance to radiotherapy.

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04. Disclosing macrophage-mediated radioresistance in triple-negative breast cancer

Authors and Affiliations

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Abstract

Triple-negative breast cancer (TNBC) is the most aggressive and radioresistant subtype, orphan of efficient target therapies. To overcome radioresistance, attention must be paid to macrophages, highly recruited upon radiotherapy (RT), modulating tumor progression. Our study aims to unravel the role of macrophages on the modulation of TNBC radioresistance and immune escape, dissecting the associated mechanisms. For this, spheroids gathering MDA-MB-231 cells and human macrophages were submitted, or not, to two RT schemes: 2.67Gy and 5.2Gy for five cumulative fractions. Then, tumor spheroids (TS) and tumor-immune spheroids (TiS) were dissociated to evaluate the impact of RT on spheroids morphology, viability and cells profile. The molecular mechanisms underlying radioresistance were explored through RNASeq, proteomics and Multiplex ELISA. Upon RT, TS disintegrated and an increase in early apoptosis and necrosis was observed, whereas irradiated TiS did not present significant differences regarding morphology and cellular viability. Strikingly, macrophages protected tumor cells from death caused by RT, potentiated cancer cells proliferation, and promoted inflammatory cytokines secretion, such as IL1Ra, IL6, CCL22, which may favor radioresistance. Moreover, GSEA revealed that, while irradiated cancer cells from TS presented severe disregulation of cell cycle, DNA replication and DNA damage response via ATR pathways, irradiated cancer cells from TiS exhibited enhanced IL4-mediated regulation of apoptosis and Wnt interactions

in lipid metabolism, both associated with the radioresistance of multiple cancers. We are now crossing the RNASeq with proteomics data, and exploring novel targets for immunomodulatory therapies that, combined with RT, abrogate macrophage-mediated radioresistance and sensitize cancer cells to improve patients treatment and prognosis.

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05. BCG vaccine activates an immediate response in macrophages within the tumor microenvironment to kill bladder cancer cells via TNF signaling

Authors and Affiliations

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Abstract

Introduction: initially developed as a vaccine to prevent tuberculosis, treatment with the BCG vaccine, based on the "Coley's-toxins" principle, is the cancer immunotherapy longest in use.

Materials and Methods: here, we set-out to develop a zebrafish-xenograft model for human bladder cancer to study in real-time and single-cell resolution how BCG modulates the earliest interactions between cancer and innate immunity.

Results: we show that BCG promotes tumor clearance and apoptosis. Mechanistically, BCG induces massive recruitment of macrophages to the tumor microenvironment and modulates their morphology and behavior towards a proinflammatory phenotype (M1-like), while also promoting macrophage fusion-like events. We demonstrate that macrophages are critical for the BCG anti-cancer effects and that tumor clearance/apoptosis is dependent on TNF signaling. As a proof of concept, we also describe how the zebrafish-xenograft model provides resolution to show specific tumoral responses to different BCG vaccine strains.

Conclusions: our work demonstrates the fundamental role of macrophages for BCG cancer immunotherapy and the potential of this unique in vivo preclinical model for the comparative testing of new candidate immunomodulators. Hence, this model not only allows to interrogate the biological mechanisms of BCG mode of action but it can markedly accelerate the pipeline for new innate-based immunotherapies.

06. type I procollagen carboxyterminal propeptide plays a role in the innate immune system dynamics and immune evasion in triple negative breast cancer

Authors and Affiliations

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Abstract

Fragments generated during production of collagen-I (col-I) are associated with desmoplasia. Desmoplasia, a process frequent in breast carcinoma, results in enhanced type I collagen deposition, and consequently, generation of C-terminal pro-peptide of pro-col-I (P1CP), a known sensitive marker for osteoblastic bone metastasis with robust prognostic value. However, the role of col-I fragments in tumoral heterotypic cell interactions and in tumor-host response has been poorly explored and may represent an area of therapeutic development. We aimed to uncover P1CP's role in tumor microenvironment dynamics regarding cancer cell invasion, metastasis formation and interaction with the innate immune system.

We conducted our studies in vitro, using a 3D spheroid model and in vivo, with zebrafish xenografts. Homospheroids were composed of different breast cancer (BC) cell lines: MDA-MB-231, MDA-MB-231 BO2 (bone organotropism) and MDA-MB-231 BrM (brain organotropism); heterospheroids were formed together with monocytic (THP-I) and macrophage-like (THP-I derived) cells.

We demonstrated that P1CP influences the metastatic potential of MDA-MB-231 cells, as shown by the increased invasion in homospheroids and metastasis formation in zebrafish xenografts. Furthermore, P1CP treatment of inflammatory macrophages co-cultured with BO2 cells led to a switch of anti-tumor macrophages to a phenotype promoting BC cell invasion. RNA-seq of these macrophages revealed a decrease in M1-like macrophages (anti-tumoral) markers and an increase in proteins characteristic of M2-like pro-tumoral macrophages. The engraftment rate of the MDA-MB-231 xenografts was also increased in the presence of P1CP, while macrophage tumoral infiltration decreased. Moreover, there was a reduction in intratumoral TNF-α positive macrophages (a classical marker for inflammatory M1-like macrophages).

This work hints at a potential effect of P1CP in switching macrophage polarization status and in cancer cell evasion from the innate immune system.

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07. New imidazole-based scaffolds for the treatment of renal cell carcinoma

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Abstract

Renal cell carcinoma (RCC) accounts for 90 to 95% of all kidney malignancies. This cancer was responsible for 403,262 cases worldwide in 2020 and 175,089 deaths in the same year, numbers that will increase dramatically until 2040. Renal cell carcinoma is frequently diagnosed in the metastatic stage (30%), requiring systemic treatment. In addition, patients have low response rates to existing therapies, a high relapse frequency after the first treatment (30%) and develop resistance to targeted therapy shortly after 6-10 months.

A virtual screening carried out on a library of compounds synthesized by the research group, allowed the identification of a selection of molecules containing the imidazole core, as potentially active in relevant biological targets for the treatment of RCC. Thus, this work reports the organic synthesis of new imidazole derivatives and their anticancer evaluation for the treatment of RCC.

Imidazo-pyridines and imidazo-diazepines were prepared from imidazolyl-pyrrolones, using previously in-house developed synthetic approaches. The optimized experimental procedures allowed the isolation of the new molecules with good yields. The imidazoles were evaluated for their anticancer activity using two RCC cell lines and also the non-neoplastic kidney cells HK2, to study the selectivity of

these new molecules. A screening of the molecules in RCC cell lines 786-O and A498 was carried out to evaluate the effect on cell viability and two imidazo-pyridines with promising anticancer potential were identified. The IC50 value (concentration responsible for a 50% cell viability reduction) of these molecules was calculated, as well as their selectivity index. Rapamycin, Cederanib and Sunitinib were used as references drugs.

In conclusion, the novel synthesized imidazoles showed low micromolar IC50 values and good selectivity indexes for RCC cells, especially when compared with the reference drugs. This promising hit molecules will be further explored for RCC treatment, addressing an unmet medical need.

08. A transcriptomic profiling reveals novel roles of Cadherin-3 in metabolic programming of glioblastoma

Authors and Affiliations

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Abstract

Introduction: Glioblastoma (GBM) is the most aggressive primary brain tumor, responsible for a poor prognosis of the patients. Previously, we established CDH3/Cadherin-3 (known as P-cadherin) as an oncogene in GBM, displaying pro-tumoral effects in vitro and in vivo, and a negative impact on the prognosis. However, the specific cellular mechanisms mediated by P-cadherin in GBM aggressiveness were still elusive.

Materials/Methods: RNA-sequencing was performed in GBM cells, including a patient-derived GBM culture and a GBM cell line, genetically manipulated to express differential levels of CDH3. Metabolism analyses included glucose and lactate quantifications and extracellular flux analyses using Seahorse XFe24 Analyzer. Expression of metabolism markers was tested in GBM cells and in in vivo tumors. Correlations between CDH3 and metabolism genes were done in GBM patients of TCGA. Survival analyses testing CDH3 combined with metabolic markers were performed with log rank test.

Results: CDH3 transcriptomic landscape showed an enrichment of various cancer-related processes, including metabolic-associated processes. In vitro functional studies showed that P-cadherin is a driver of the classic basal metabolism of each specific cellular model. In GBM samples, CDH3 was positively and inversely correlated with a panel of glycolysis and mitochondria/OXPHOS-associated

genes, respectively. In GBM patients presenting a glycolytic-enriched gene expression signature, CDH3 high expression presented prognostic value by identifying a group of patients with shorter overall survival.

Conclusions: P-cadherin influences various cellular and molecular mechanisms, including effects on energetic metabolic-related pathways, a classic hallmark of cancer, impacting the survival of GBM patients with a particular glycolytic signature. These results open novel opportunities for stratification of specific prognostic groups of patients, and potential new therapeutic possibilities.

09. Targeting Tumour microenvironment molecules as a Potential Therapeutic Approach for Colorectal Cancer Treatment.

Authors and Affiliations

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Abstract

Human colorectal cancer (CRC) is an aggressive cancer that kills over 900,000 people worldwide, including more than 16,500 in the UK annually. The main reason for this is that the current testing and predicting models/technologies do not fully replicate the complexity of the tumour microenvironment (TME) and the spectrum of secreted molecules around tumours in patients. Therefore, to evaluate the complexity of the environment-mediated drug response, new model systems must represent the different cell-cell interactions and communications mediated by secreted molecules around tumours in patients.

Emerging research has highlighted the significance of SPOCK1, a member of the secreted protein acidic and rich in cysteine (SPARC) family, in reprogramming the extracellular matrix (ECM) and promoting epithelial-mesenchymal transition (EMT) in various cancers, including CRC. SPOCK1 has been shown to affect cancer cell adhesion, cell-matrix interactions, cell proliferation, invasion, apoptosis, and cancer recurrence.

In this study, our objective is to disrupt ECM/EMT by targeting SPOCK1 and downstream signalling pathways such as Wnt/β-catenin and matrix metalloproteinases (MMPs) in colorectal cancer. These findings suggest that interfering with SPOCK1 or the SPOCK1 pathway could be a promising strategy to impede ECM/EMT processes and subsequently impact tumour progression. Notably, to the best of our knowledge, the targeting of SPOCK1 remains unexplored, with only the Apigenin (API), which has been shown to reduce SPOCK1 expression in vitro in prostate cancer cells.

10. Synthesis of chromene[2,3-b]pyridines and characterization of their anticancer potential for the treatment of breast cancer

Authors and Affiliations

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Abstract

Breast cancer (BC) is the most diagnosed cancer worldwide and the leading cause of cancer-related death in women.

In recent years, efforts have been made to investigate new treatments that have improved the survival rates of patients with BC. However, the serious adverse effects of some of the drugs in clinical use and the emergence of resistance to these drugs require the search for new treatment strategies and the development of new drugs.

The research group has focused on the synthesis of new compounds derived from the chromene structure to be used as therapeutic agents. Chromenes are privileged molecules in medicinal chemistry, with a variety of natural and synthetic derivatives that exhibit diverse pharmacological activity.

This work presents the synthesis and evaluation of new chromene[2,3-b]pyridine derivatives as anticancer agents for the treatment of breast cancer.

Functionalized 2H-iminochromenes, previously obtained from the reaction of different salicylaldehydes with malononitrile dimer, were used as precursors for the synthesis of chromene[2,3-b]pyridines. The use of our optimized synthetic approach allowed us to obtain a library of new chromeno[2,3-b]pyridines, with excellent yields. The new molecules prepared were evaluated for their potential anticancer activity in BC cell lines (MCF-7, Hs578t and MDA-MB-231) and in the non-neoplastic line MCF-10A. A screening of the molecules obtained from the three cell lines was carried out to

evaluate the effect on cell viability and it was possible to identify three hit molecules with promising anti-cancer potential. The IC50 value of these promising molecules was calculated, as well as their selectivity index. The effects of these chromenes on cancer aggressiveness characteristics were also evaluated.

The IC50 values in the low micromolar range, the ability to inhibit cell proliferation, induction of cell cycle arrest and apoptosis, combined with the safe profile demonstrated, make these molecules very interesting for therapeutic application in BC.

The design and synthesis of more potent chromene[2,3-b]pyridine derivatives and complementary biological studies, such as determination of the mechanism of action and ADMET studies, are under development in the research group.

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11. Development of virtual screening protocols for the design of novel KDM4C inhibitors for the treatment of triple negative breast cancer

Authors and Affiliations

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Abstract

Breast cancer is the leading cause of cancer related death in women worldwide, with triple-negative breast cancer (TNBC) being the most aggressive subtype. TNBC patients respond poorly to existing therapies, with high relapse and mortality rates, highlighting the need for new therapies. Lysine-specific demethylase 4C (KDM4C) has been identified as a promising epigenetic target, responsible for several tumorigenic processes that contribute to the aggressive phenotype of TNBC. The combined use of computational biochemistry, organic synthesis and molecular biology methods has proven to be a powerful strategy for the rational design of new therapies. This work describes the first computational step of a multidisciplinary work, aiming the development of selective and potent KMD4C inhibitors.

In the Protein Data Bank, 6 experimental structures of KDM4C were selected. The ability of 4 scoring functions to reproduce the experimental poses was evaluated by protein-ligand docking with the GOLD software. In the ChEMBL database, 30 KDM4C inhibitors with IC50 values below 100 nM were identified. For each, 50 decoys were generated, resulting in a training set of 30 actives and 2000 decoys. The performance of different docking protocols in discriminating active molecules from decoys was evaluated by calculating the enrichment factor at 1% (EF1%) and the area under the curve (AUC). The protocol/structure combinations with the highest discriminative ability in active/decoy recognition and accuracy in predicting the structures of the complexes were selected.

The best results were obtained with the 5FJH and 5FJK structures, and with the ChemPLP and ASP scoring functions. The 5FJK/ChemPLP combination presented an AUC of 70.2% and an EF1% of 6.8, while the 5FJH/ASP and 5FJH/ChemPLP combinations resulted in an AUC of 83.5% and 74.7%, respectively, and an EF1% of 6.8 for both.

We obtained 3 virtual screening protocols that will be used to screen several commercial and in-house libraries of molecules to identify potential KDM4C inhibitors.

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12. Therapy-induced immunomodulation in breast cancer allografts treated simultaneously with CDK4/6 and RANKL inhibitors

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Abstract

The receptor activator of nuclear factor kB (RANK) pathway is the key regulator of bone physiopathology and progesterone-induced breast carcinogenesis, and is associated with breast cancer (BC) aggressiveness. Recently, we have disclosed a link between RANK signaling and resistance to CDK4/6 inhibitors (CDK4/6i) plus endocrine therapy, the current standard of care for metastatic luminal BC. Moreover, we have found that the genetic or pharmacological inhibition of RANK pathway improves response to CDK4/6i-based therapy, in luminal BC and triple-negative BC (TNBC). It has been shown that loss of RANK signaling in mouse or clinical BC samples has anti-tumoral immunomodulatory effects; and that CDK4/6i themselves can boost anti-tumor immune responses. Therefore, we aimed to assess if therapy-induced immunomodulation improves the efficacy of targeting CDK4/6 and RANKL in BC. With this in mind, we used the Rb-proficient and RANK-positive TS/A-E1 and 4T1 syngeneic ectopic models of luminal BC and TNBC, respectively. Tumor-harboring mice were treated with Palbociclib (CDK4/6i), OPG-Fc (RANKLi) or both. Tumor growth, proliferation index and immune cells' infiltrate were compared at sacrifice. We found that combination therapy was more effective than monotherapy in preventing tumor growth; and that RANKLi induced an anti-tumor immune response. In OPG-Fc-treated groups, leukocytes (CD45+), lymphocytes (CD3+) and M1-like (iNOS-positive) macrophages increased over-time; whereas macrophage (F4/80+) and neutrophil infiltration (Ly6G+) were reduced. CD8+ T cells only increased in the combination group. Overall, our data supports that RANKLi can be an important add-on to CDK4/6i, in both luminal and TNBC, warranting future clinical studies. Animal experiments licensed by DGAV (#005132-2020) and conducted in accordance with approved guidelines, at the Rodent Facility of iMM-JLA. Work partially funded by PTDC/MED-ONC/28636/2017 and SFRH/BD/139178/2018 (FCT/MCTES).

13. Development of anti-nucleolin antibody against Triple-Negative Breast Cancer

Authors and Affiliations

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Abstract

Introduction: Triple-Negative Breast Cancer (TNBC) is a clinically aggressive cancer, with the highest mortality after 6 months of diagnosis, metastatic potential, and risk of relapse amongst breast tumors. The main challenge regarding TNBC treatment stands from the lack of cell surface expression of the most common molecular markers and therapeutic targets of breast cancers. The present work aims at optimizing an anti-nucleolin VHH-Fc antibody previously developed by our group, to increase nucleolin-mediated Antibody-Dependent Cellular Cytotoxicity (ADCC) activity against nucleolin-overexpressing and TNBC cells.

Materials and Methods: Five mutant antibody clones were selected using an affinity maturation strategy developed by us. Binding to nucleolin-overexpressing cells was assessed by flow cytometry. Binding kinetics and affinity was studied in live cells using LigandTracer® technology. ADCC was measured using xCelligence Real-Time Cell Analyzer, upon incubating target nucleolin-overexpressing cells with the antibodies and effector human Peripheral Blood Mononuclear Cells (PBMCs).

Results: Two antibodies demonstrated improved binding, affinity, and nucleolin-mediated ADCC against nucleolin-overexpressing and TNBC cells, relative to the original antibody.

Conclusions: Overall, our results corroborate the affinity maturation approach established by us, and culminated in the development of two anti-nucleolin antibodies capable of nucleolin-mediated ADCC. They confirm the potential of nucleolin as a novel therapeutic target in TNBC, and of anti-nucleolin antibodies as a targeted therapy against the disease.

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14. Intravenous administration of poly(I:C)+resiquimod reprograms tumor associated macrophages in solid tumors and prevents metastasis

Authors and Affiliations

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Abstract

Introduction and Objectives: Tumor associated macrophages (TAMs) are a key drivers of immunosuppression in solid tumors and support cancer progression, metastasis and recurrence after treatment. In the last years, we have developed immunomodulatory therapeutic strategies to counteract macrophage pro-tumor activities. Here, we investigated the antitumoral efficacy of poly(I:C)+R848 intravenously administered in murine solid tumors.

Materials and Methods: Agonists of TLR3 and TLR7/8 (Poly(I:C)+R848) were intravenously (i.v.) injected in the tail vein. Orthotopic models of lung cancer (CMT167) and fibrosarcoma (MN/MCA) were prepared using fully immunocompetent C57BL/6 mice. In addition, immunocompetent Balb/c mice were implanted with 4T1 cancer cells for the orthotopic breast cancer model. Lung metastasis were evaluated by Bouin fixation or H&E staining. Tumor-infiltrating leukocytes were evaluated by flow cytometry and multispectral immunophenotyping analysis. Cytokines in blood circulation were evaluated by Luminex.

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Results: The i.v. administration of the poly(I:C)+R848 combination significantly reduced the growth of primary tumors (lung, breast and fibrosarcoma). Treated mice presented an increase in circulating pro-inflammatory cytokines, such as TNF-alfa and IL-6. The tumor microenvironment of treated mice had higher infiltration of macrophages showing their reprogramming towards an M1-antitumoral phenotype, characterized by increase in CD86 and decrease in Arginase1. Furthermore, in the orthotopic lung cancer model, a significant reduction of CD206 was observed in the interstitial macrophages. Notably, lung metastasis was prevented by i.v. poly(I:C)+R848 treatment both in the fibrosarcoma and in the breast cancer models.

Conclusions: Intravenous administration of poly(I:C)+R848 showed the capacity to reprogram TAMs to reduce tumor progression and prevent metastasis in a variety of pre-clinical murine cancer models.

15. Establishing a Sub-Saharan cancer cell line panel by conditional reprogramming

Authors and Affiliations

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Abstract

Introduction: Cancer is an increasing public health burden, including in sub-Saharan Africa (SSA), where incidence will double in the next 20 years. These rates ask for a better understanding of cancer across SSA regions, which display variable genetic and environmental features. This project aims to maximize gains from a careful collection of SSA cancer samples, establishing a much-needed, reliable and omics-characterized SSA cancer cell line panel propagated by Conditional Reprogramming (CR).

Methods: Fresh tumour samples were collected from SSA ancestry patients in surgeries at IPO-Porto, Hospital Santa Maria-Porto and Hospital Garcia-Orta-Lisbon, totalling 5 cases: 2 gastric, 1 salivary glands (SG), 1 breast (B) and 1 endometrium. The CR was done by culturing samples on feeder cells: irradiated mouse fibroblasts (3T3-J2, AddexBioP0011008) with gamma rays (70Gy). The cells were monitored by bright-field microscopy, and characterized by autosomal STR DNA profile, immunofluorescence (cytokeratins CK7 and CK20) and assessment of the cell cycle by FACS with propidium iodide staining.

Results: We managed to have culture growth in 2 samples, namely SG (passage 14) and B (passage 6). The STR DNA profiles were unique, with absence of the microsatellite instability phenotype. Immunofluorescence results confirmed the tumour tissue of origin: CK7+/CK20- for SG tumour.

Conclusions: We were able to establish the CR technique and to initiate an efficient pipeline for the collection of fresh tumour samples from SSA patients in three Portuguese hospitals. We have still to fine-tune the method, but we are within the described 30-60% success rate. Moreover, the characterization methods suggest that the characteristics intrinsic to the tumour tissues were maintained. In a future perspective, the established cancer cell lines reflecting SSA diversity will be a useful resource, namely for a high-throughput screening of drugs.

16. Extracellular vesicles (EVs) as nanocarriers of anti-tumor immune response

Authors and Affiliations

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Abstract

Introduction: A promising approach in treating glioma (WHO grade IV) is the use of oncolytic viruses (OVs). However, infection efficacy in patients is unsatisfactory and does not match the ex vivo efficacy. Thus, activating the immune system following OV therapy is crucial to its effectiveness. Our approach uses extracellular vesicles (EVs) as nanocarriers, instigating an anti-tumor immune response as they contain tumor-specific antigens secreted by infected cancer cells.

Material and Methods: Patient-derived glioblastoma stem-like cells (GSCs) (n=6) were infected ex vivo with OV (at MOI 0.1). The cellular transcriptome and protein secretome were then analyzed by gene microarray (n=21393) and mass spectroscopy (n=3569). Ex vivo peripheral blood mononuclear cells (PBMC) activation tests (by RT-PCR and flow cytometry) were performed with protein EV fraction obtained from GSCs after therapeutic OV infection.

Results: Bioinformatic analysis revealed that OV infection altered the transcriptome (n=21393, $p<0.05$, FC>2, up=704, down=350) of GSCs and the protein secretome (n=3569). EV fraction of virus-free secretome upon OV infection analyzed by mass spectrometry (n=3569) was enriched in immune response co-stimulatory proteins (CD86, CD48, CXCL10) while co-inhibitory proteins were reduced (CD276, PVR, TNFRSF14).

Ex vivo tests of PBMCs in the presence of these EVs showed an increase in immune cell activity (as measured by levels of IFN-γ, TNF-α, IL-2, IL-4, IL-6). Indeed, expression analysis of cytokines such as IFN-γ and TNF-α showed a positive correlation with PMBC cell activity, associated with increased expression and secretion of CD86 co-stimulatory molecule and depleted CD276 co-inhibitor secreted by OV-infected GSCs.

Conclusions: Analysis of the GSCs secretome in response to OV therapy provided insight into the complexity of the immune response of glioma, demonstrating that the secreted EVs have immunostimulatory properties and thus indicating their therapeutic value.

17. An alternative mechanism of action for anti-angiogenic therapies: an immunotherapy-like treatment around the corner?

Authors and Affiliations

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Abstract

Macrophages are plastic cells that play a key role in the tumor microenvironment (TME) of most tumors. Depending on the tumors, macrophages can have a pro- or anti-tumoral function. In breast cancer tumors, a high infiltration of macrophages is often correlated with a poor prognosis.

Among the several molecules overexpressed in the TMEs, VEGF-A is often upregulated in a variety of tumors and is one of the most potent pro-angiogenic factors. Although VEGF signaling is mostly related to angiogenesis, VEGF-A has also been shown to regulate tumor cell survival and migration. However, research is mainly focused on the impact of anti-angiogenic therapies on endothelial cells, not exploring their effect on other cell populations.

Here, we investigated the impact of bevacizumab, an anti-VEGF-A therapy, on innate immune cell populations present in the TME by using zebrafish larvae xenografts, where the influence in the TME can be readily analyzed. By using a bevacizumab sensitive breast cancer cell line, in which bevacizumab impairs angiogenesis and shrinks tumor size, we show that bevacizumab can modulate the innate immune cell populations present in the TME. Bevacizumab can switch the tumor-associated macrophages towards a pro-inflammatory M1-like phenotype. Strikingly, depletion of macrophages, genetically or chemically, leads to the same phenotype as bevacizumab, i.e reduction of tumor size and impairment of angiogenesis, suggesting that VEGF-A is involved in breast cancer cells survival through recruitment of tumor-associated macrophages, independently of angiogenesis.

Currently, we are disentangling the triad – tumor cells, macrophages, and endothelial cells – by using in vitro and in vivo approaches where we restrict the assay to the populations of interest – tumor cells and macrophages - to fully test if bevacizumab anti-tumor activity is via macrophages.

If so, our work may open new avenues for a wider usage of bevacizumab as macrophage immunomodulator therapy.

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18. Chromene-based molecules: privileged scaffold for oncology drug development

Authors and Affiliations

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Abstract

Breast cancer (BC) is the most diagnosed cancer worldwide and the main cause of cancer-related deaths among women. Triple-negative breast cancer (TNBC) represents 15-20% of all breast cancers, considered the most aggressive subtype and an unmet medical need. Chemotherapy, surgery and radiation are the current standard therapeutic options, but present very limited results and induce severe side-effects. Metastasis formation, the low response to existing therapy and the high rate of relapse (50% of early-stage patients) are major problems in TNBC. Also, 37% of early-stage and 90% of late-stage patients experience a high 5-year mortality rate. Thus, there is an urgent medical need for new effective and safe approaches.

Chromene-based scaffolds have been explored as medicinal agents and their biological activity has been extensively studied. Chromenes have been reported with significant anticancer properties, targeting several molecular pathways involved in cancer progression. New chromenes were synthesized in-house, using optimized reaction conditions. The anticancer potential of the novel molecules was investigated in vitro and in vivo using different BC cell lines. The efficacy and safety profiles of the top lead molecules was studied, including their effect on several cancer aggressiveness features. The lead molecules revealed potent in vitro anticancer activity and selectivity towards cancer cells, the capacity to induce regulated cell death, cell cycle arrest and to impair cell proliferation and invasion.

Efficacy studies using the most promising molecules in the ex vivo CAM model revealed significant tumor regression and reduction in blood vessel formation. Toxicity was studied in vivo using the Caenorhabditis elegans and mouse models and no adverse effects were detected, indicative of a safe preclinical profile.

The present study provides evidence for the effectiveness and safety of new drug candidates for TNBC therapy, offering a new hope for BC patients suffering from this aggressive disease.

19. ASPA as a new regulator of cancer-associated fibroblasts phenotype and its implication in cancer

Authors and Affiliations

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Abstract

The crosstalk between cancer cells and the tumor microenvironment (TME) plays a critical role in the acquisition of molecular and cellular features underpinning tumor progression. Cancer Associated Fibroblasts (CAFs) are the major population of the TME and their contribution to most of the hallmarks of cancer is gradually emerging. CAFs present a pathologically activated phenotype that promotes extracellular matrix remodeling and pro-tumorigenic signaling to cancer cells. CAFs may also present metabolic adaptations to fuel cancer cell growth. However, how metabolic reprogramming assists in the pathological activation of fibroblasts, and its interplay with signaling and transcriptional rearrangements is not well defined.

To identify new stromal modulators of cancer progression, gene expression datasets of cancerous and normal stroma from human breast, ovary, colon, prostate, and lung tissues were analyzed. Consistent up or down-regulated genes were shortlisted based on their association with disease-free survival, and further investigated using genetic manipulation in murine and human models, molecular characterization, and functional in vitro and in vivo assays.

These analyses revealed that the enzyme Aspartoacylase (ASPA) is consistently downregulated in CAFs of different tumor types, and it is correlated with a poorer prognosis in human breast and prostate cancer. Modulation of ASPA expression in normal fibroblasts or CAFs affect their levels of CAF marker expression, extracellular matrix remodeling capacity and crosstalk to cancer cells. Our results show that ASPA expression is downregulated by the transcription factor TAZ downstream of TGFb signaling. In addition, ASPA downregulation may negatively affect the intracellular pool of acetate, potentially affecting histone acetylation and epigenetic reprogramming.

Understanding the role of ASPA in CAFs may illuminate the metabolic crosstalk between tumor and stroma, and how these perturbations lead to the emergence of a pro-tumor CAF phenotype.

20. New approach to target triple negative breast cancer brain metastasis.

Authors and Affiliations

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Abstract

Triple negative breast cancer (TNBC) is the most aggressive breast cancer (BC) subtype, being prone to the development of brain metastases (BM), whose treatment is challenging as the blood brain barrier (BBB) hinders the delivery of pharmaceuticals. Additionally, Cancer Stem Cells (CSCs) of TNBC are involved chemoresistance and metastization, with evidences of the Wnt/β catenin signaling pathway being overexpressed and the Frizzle receptor (FzD) family constituting a promising new target. We propose an anti FzD antibody (FzDa) conjugated with two peptides – a BBB peptide shuttle (BBBpS) and an anticancer peptide (ACP), to target CSCs simultaneously in the primary tumor and BM. First, we characterized BC cell lines by flow cytometry verifying that TNBC (MDA MB-231 and BT 20) have more CSCs (99% and 5%, respectively), when compared to human-epidermal growth factor receptor-2 (HER2+)(SkBr3; 0%). TNBC spheroids resulted in similar % of CSCs for MDA-MB-231 (99%), and an increased % (89%) for BT-20. The activity of the compounds BBBpS, ACP, ACP-BBBpS, FzDa and FzDa-BBBpS was determined in MDA MB 231 and brain endothelial cells (HBEC-5i), using cytotoxicity (reazurin reduction assay), cell migration (wound healing), invasion and BBB translocation assays. Previous results from our lab shown that the BBBpS was nontoxic and capable of crossing an in vitro BBB model, while the ACP showed a preferential action towards MDA MB 231. Here, we demonstrate that FzDa and FzDa-BBBpS have no cytotoxicity towards MDA MB 231 and HBEC-5i, hence not posing safety concerns. The ACP, ACP-BBBpS and FzDa-BBBpS efficiently inhibited wound closure in MDA-MB-231. These migration results are being corroborated by a transwell migration assay. Considering the translocation, FzDa-BBBpS demonstrated a 3.5-fold increase translocation compared to FzDa. Together, the impairment of CSCs migration and increased BBB translocation in vitro are promising results, suggesting that these constructs could be used as new drug leads.

21. Carbon Quantum Dots and their application in breast cancer photothermal therapy and imaging: an early study

Authors and Affiliations

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Abstract

Triple-negative breast cancer has no target therapies options, prompting the search for alternatives due to suboptimal clinical outcomes. Photothermal therapy (PTT) is a targeted and non-invasive treatment, based on the principle of converting light energy into heat. Temperatures rises (39 to 45 °C) inhibit DNA/RNA synthesis and repair, discriminatively killing temperature sensitive cancer cells. PTT uses photothermal agents, like carbon quantum dots (CQD). Specific criteria were required: sub-10 nm lateral size, water stability, photoluminescence (PL), photothermal conversion and biocompatibility. Due to their representation of normal breast tissue, and the ease of comparison with cancer models, MCF10A cells were used.

Our innovative method uses electrochemical exfoliation to produce CQD. A custom apparatus with a graphite rod (cathode) and a metal rod (anode) in water was used. A 1000 V potential and 60 mA current consumes the graphite rod. Subsequently, large debris are removed using syringe filters with 200 nm pore size. PTT tested CQD dispersions (100, 150, and 250 µg·mL-1) under custom LED near infra-red-light irradiation (30 min, 1 W·cm-2). For biocompatibility we seeded 2.5 × 104 cells/well in a 48 well-plate 24h prior to incubation with CQD (100, 150, 250 µg·mL-1), media (positive), 10% DMSO (negative), for 3 days.

Electron microscopy showed 8-10 nm lateral sizes. Surface charge was -33.3 mV by zeta potential. Raman spectroscopy displayed D, G, and 2D bands, contributing to PL. PL measurements indicate excitation peak at 340 nm, and emission peak at 448 nm. Photothermal assays increased temperature in proportion to concentration and irradiation time; the highest temperature reached was 51ºC. Viability was determined by Presto blue assay and all concentrations showed viability above 70%, complying with ISO 10993-5:2009(E).

Our findings show that CQD, produced by a new electrochemical exfoliation method, have a potential to be used as a photothermal agent for PTT, as well as for PL imaging.

22. Microbiota: a new player in breast tumour microenvironment?

Authors and Affiliations

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Abstract

Recent studies suggest that tumour microbiota may correlate with breast cancer (BC) incidence and progression, including response to therapies. Geographical factors highly influence the microbiota and, thus far, there is no characterization of BC microbiota in patients residing in Portugal, where BC represents 7000 new cases and 1800 deaths yearly. Therefore, we aim to characterize the Portuguese BC microbiota in primary and metastatic tumour samples.

The research methodology involves qRT-PCR and 16S rRNA sequencing to define potential microbial signatures. Conventional and fluorescent immunohistochemistry (IHC) support the definition and optimization of microbiota biomarkers suitable for multiplexed imaging (CODEX technology, PhenoCycler platform). We will address the crosstalk between the microbiota and host (tumour and immune) cells in situ with multiplexed imaging.

BC surgical samples after neoadjuvant therapy show detectable bacterial 16S rRNA. By qRT-PCR, we observe that BC samples have a specific distribution of bacteria phyla (Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria) and higher bacterial load than environmental controls. In addition, lipopolysaccharide was visualized by conventional microscopy in BC samples.

The last two decades have outstanded the importance of the tumour microenvironment in cancer progression and treatment response. We are using multiplexed imaging to explore immune myeloid and lymphoid cells, vasculature and tumour/epithelial cells in BC samples. To do so we have developed a PhenoCycler panel that will be expanded with our under-development bacterial biomarkers. Preliminary data with fluorescent IHC show co-localization of bacteria with both tumour/epithelial cells and stroma cells in situ, suggesting an interplay between the microbiota and the host tumour and immune cells.

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23. The obesogen tributyltin programs periprostatic adipose tissue to a cancer driven phenotype

Authors and Affiliations

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Abstract

The "obese" periprostatic adipose tissue (PPAT) has been implicated in the aggressiveness of prostate cancer (PCa), with the dysregulation of its secretome (adipokines, chemokines, metabolites and growth factors) significantly contributing to a tumour-promoting microenvironment, and accelerating the progression of disease. Evidence also has linked obesity with environmental influences, namely, the action of the so-called obesogens, i.e. endocrine-disrupting chemicals capable of dysregulating adipose tissue and promoting fat accumulation. Herein, we hypothesize that obesogens can dysregulate PPAT to a cancer-driven phenotype. Rat PPAT was exposed to the model obesogen tributyltin (TBT, 100 nM) for 48 h. Then, PNT1A non-neoplastic human prostate cells were co-cultured with the TBT-treated PPAT or their secretome (conditioned media assays) for 24 h. PPAT and its secretome were evaluated by histological analysis and colorimetric assays. PNT1A cell fate was analysed by MTT assay, Ki-67 immunocytochemistry, caspase-3-like activity, and scratch assay. TBT promoted adipocyte enlargement not affecting viability, which was accompanied by an altered secretome. TBT highly increased free-fatty acids, glucose, leptin and CCL7 content in the PPAT culture media while decreasing adiponectin and tumour necrosis factor-alpha, together with higher levels of lipid peroxidation. Importantly, TBT-treated PPAT and its secretome augmented the viability, proliferation, and migration of PNT1A cells and reduced the apoptotic rate. This study first demonstrated the relevance of PCa-obesity-environment triad, bringing evidence about the dysregulation of the oxidative, inflammatory, and metabolic status of PPAT in response to TBT in supporting a pro-tumorigenic microenvironment, being a driving force in prostate carcinogenesis.

24. Nucleolin as a CAR-T cell target for solid tumors – brief notions into anti-nucleolin CAR-T cells manufacturing and in vitro testing

Authors and Affiliations

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Abstract

Introduction: Genetic modification of human T cells to express chimeric antigen receptors (CAR-T cells) redirects their antitumor activity towards pre-defined tumor antigens. Despite the success in hematological cancers, their use in solid tumors remains a challenge, due to inefficient tumor access and strong immunosuppression. In this context, nucleolin, a membrane-nucleus shuttling protein, has been reported to be overexpressed at the surface of cancer and endothelial cells from tumor vessels, in different solid tumors. Accordingly, anti-nucleolin VHH nanobodies, previously designed by our team, showed high binding capacity towards nucleolin-positive cancer cell lines, highlighting nucleolin as a potential target for immunotherapy, namely CAR-T cells. Therefore, this work focuses on the first steps of anti-nucleolin CAR-T cells generation and in vitro testing, against solid tumor cell lines.

Materials and methods: Peripheral blood mononuclear cells were isolated from healthy donors, activated with CD3/CD28 agonists, and transduced with produced CAR-encoding lentivirus. T cells with the highest transduction rate were sorted, expanded and co-cultured with target cells in different E(effector):T(target) ratios. CAR-T cells cytotoxicity was assessed by impedance-based assays. T cells transduced with a mock CAR were used as controls.

Results: Transduction efficiency ranged from 20-40%, and more than 80% of the CAR-T population were CD4+ T cells. In real-time impedance-based coculture assays, higher E:T ratios were associated with higher levels of target cell death, and differences in cytotoxicity of both test and control CAR-T cells were observed.

Conclusions: Overall, this work provides insight on anti-nucleolin CAR-T cells generation and evaluation.

25. Dendritic cells loaded with cancer-testis antigens to halt triple-negative breast cancer

Authors and Affiliations

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Abstract

Introduction: triple-negative breast cancer (TNBC) lacks specific cell markers; however, it has been reported that TNBC cells display cancer-testis antigens (CTAs), which are expressed just in cancer cells. Hence, we hypothesised that a vaccine composed of dendritic cells (DCs) loaded with CTAs can prevent TNBC. To achieve this, we aimed to determine the best DC-loading strategy and identify CTA levels in TNBC cells.

Methods: human monocytes were isolated from buffy coats of healthy donors and differentiated into monocyte-derived DCs using conventional (CONV) and proprietary differentiation (PDC) cocktails. These cells were tested for eGFP-coding mRNA transfection by nucleofection and lipofection and improvement of long peptide cross-presentation using a transduction solution (TS). The presence of a specific CTA (CTA-A) was evaluated in TNBC cell lines (MDA-MB-231 and 4T1) by western blotting.

Results: both mRNA transfection strategies resulted in eGFP expression in DCs, with higher expression levels observed after nucleofection in CONV-DCs. However, PDC-DCs showed better cell viability. Regarding DC loading with a long peptide, TS appears to improve specific cancer cell death by CD8+ T cells primed with PDC-DCs. CTA-A expression was detected in MDA-MB-231 and 4T1 cells.

Conclusions: the most efficient method for mRNA transfection was nucleofection with CONV treatment. In addition, TS appears to improve DCs' cross-presentation by long peptides in PDC-DCs. Nevertheless, further studies are needed to better understand the optimal loading procedure. Finally, CTA-A may be used as a target to load DCs in TNBC clinical settings.

26. Can chemotherapy response be predicted by studying inflammatory cells in the tumour microenvironment of pancreatic cancer?

Authors and Affiliations

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Abstract

Introduction: Pancreatic cancer is a very aggressive cancer, being pancreatic adenocarcinoma (PDAC) the most common type. The most effective method to treat this disease is surgical removal of the tumour. However, most patients are not suitable for surgery and are treated with chemotherapy (CTx), which is unsuccessful in most cases. Several studies have been conducted to find markers to determine the CTx response in PDAC patients, but it still remains unreachable.

The aim is to study whether it is possible to predict CTx response in PDAC patients through the quantification, analyses of spatial distribution, and functional status of inflammatory cells in the tumour microenvironment (TME) of PDAC.

Materials and Methods: Formalin-fixed paraffin-embedded (FFPE) blocks of 34 PDAC patient biopsies and surgical specimens (some with paired pre-treatment biopsy and post-treatment surgical specimen to compare how CTx modifies the immune infiltrates) were used. To characterise the TME, multispectral microscopy was used, analysing two multiplex immunofluorescence panels with CD3 (T cells), CD4 (helper T cells), CD8 (cytotoxic T cells), FOXP3 (regulatory T cells), CD56 (natural killer cells), CD68 (macrophages), CD80 (anti-tumoural macrophages), CD206 (pro-tumoural macrophages), and cytokeratin 18 (epithelial cells).

Results: Significant differences were observed in the expression of the markers throughout the different compartments (intraepithelial, intratumoural stroma and periphery stroma), being all mostly expressed in the intratumoural. Comparisons between both tissues and compartments also demonstrated meaningful differences. Kaplan-Meier curves assessed the prognosis and differences were also observed. A correlation between the number of cells in patients who had a local complete response after CTx and those that did not was also made.

Conclusions: With these results, it is still not possible to predict response to CTx, but the presence of specific inflammatory cells may be associated with a better response.

27. Characterization of the colon adenocarcinoma microenvironment with a focus on natural killer cells

Authors and Affiliations

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Abstract

Introduction: Colorectal Cancer (CRC) is the third most common cancer worldwide. In patients with advanced CRC, standard treatment strategies have shown low effectiveness. More recently, Natural Killer (NK) cells have appeared as an alternative to T-cells as adoptive cell therapy due to their lesser secondary effects, strong cytotoxicity, and tumour microenvironment (TME) regulatory capacity. The main goal of this project is to evaluate the immune infiltrates in CRC tissue specimens, with a focus on NK cells, using multiplex immunofluorescence.

Material and Methods: Formalin-fixed paraffin-embedded tumour specimens from 10 patients who underwent surgery for CRC were used. Multispectral microscopy was used to quantify and analyse the spatial distribution of NK cells, their subtypes and functionality, using antibodies from different animal sources. After initial Mplex optimization, regions of interest were acquired for each specimen with a multispectral camera. The images were generated with the Nuance software and later analysed using the Fiji/ ImageJ software. To evaluate the activation state of NK cells in the CRC TME a Mplex was developed, with the following biomarkers: NCAM1 (NK Cells), CD16 (cytotoxic NK cells), NKG2D (activated NK cells), CD3 (T cells) and Pan-Cytokeratin which was used to label the tumour cells to localize the immune cells with respect of the neoplastic glands. Different tumour compartments: stroma on the periphery of the tumour, stroma between the tumour glands and intraepithelial were analysed and correlated with clinicopathological patient characteristics.

Results: The results obtained demonstrate that NK cells there was a significant association between high number of NK cells and low stages of tumour progression. Notably, lower number of infiltrating NK cells in CRC is associated with a higher risk of mortality.

Conclusions: At early stages NK cells infiltrating tumours underscores the potential role of these innate immune cells in restraining tumour growth and suppressing metastasis.

28. The role of extracellular vesicles glycosylation in tumour-adipose tissue communication

Authors and Affiliations

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Abstract

De novo synthesis of aberrant glycans by gastric cancer (GC) cells is associated with patients' poor prognosis. Our group previously demonstrated that GC cells displaying short O-glycans induced weight loss in mouse models, critically impacting their survival. In addition, we have also detected these aberrant glycans in GC-derived extracellular vesicles (EVs). Therefore, we hypothesized that EVs carrying short O-glycans have a role in tumour-adipose tissue crosstalk.

EVs from differentially O-glycosylated GC cells were isolated, fully characterized and their uptake by adipocytes was evaluated by flow cytometry. The impact of GC cells and EVs on adipocytes metabolism was assessed by evaluating the transcriptomic level of specific genes by RT-qPCR. The metabolomic profile of these GC cells was also studied. Finally, the capacity of activated adipocytes to modulate the phenotypic behaviour of GC cells was evaluated by co-culture assays.

Our results showed that aberrant O-glycosylation affected GC cells metabolism. Furthermore, EVs carrying short O-glycans were more internalized by adipocytes, which affected the transcriptomic level of genes involved in adipocyte metabolism. Additionally, we found that adipocytes could secrete factors that induced an increased migration capacity of GC recipient cells.

This study demonstrates that adipocytes preferentially uptake GC EVs carrying aberrant O-glycans, which affected the transcriptomic level of certain metabolic genes. The feedback impact of adipocytes on the biological behaviour of GC cells was also proven. We will further explore the mechanism sustaining this tumour-adipose tissue crosstalk both in vitro and in vivo.

29. Role of HSF1 in the generation of aggressive tumour-microenvironments by cancer associated fibroblasts (CAFs)

Authors and Affiliations

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Abstract

Cancer associated fibroblasts (CAFs) can promote cancer progression and dissemination and represent a critical target to modulate the tumour microenvironment (TME) for clinical benefit. Although it is accepted that CAFs arise from normal resident fibroblasts, the mechanisms underlying their permanent activation are not fully understood. HSF1 has been implicated in the modulation of CAF pro-tumor functions independently of its role in regulating heat shock responses, a mechanism also observed in cancer cells. The correlation with clinic-pathological features of aggressiveness, both in cancer and stromal cells, presents HSF1 as a crucial factor in the transcriptional reprogramming of cancer stroma from tumor-repressive to tumor-supportive phenotypes.

To investigate how HSF1 elicits pro-tumor programs in CAFs we employed CAFs and Normal Fibroblasts (NFs) from a murine model of breast cancer, that were extensively characterized at molecular, functional and biological levels after genetic manipulation of HSF1.

Here we show that HSF1 specifically modulates the ability of CAFs to contract collagen gels and to migrate, suggesting a novel role of HSF1 in controlling cytoskeletal features. Also, inhibition of HSF1 attenuates the pro-tumorigenic influence of CAFs over cancer cell proliferation. Gene expression analyses of CAFs after Hsf1 inhibition reveal potential mechanisms that strongly associate HSF1 with pro-tumorigenic phenotypes in CAFs, as well as novel cellular/molecular functions such as cell cycle control, regulation of stress responses and immune modulation. HSF1 has minimal transcriptional effects in NFs, suggesting that HSF1 may be capable of hijacking additional programs as a result of fibroblast activation.

To conclude, we present HSF1 as a critical transcription factor in fibroblast activation, which may be modulating new tumour promoting functions in CAFs. Thus, targeting HSF1 activity may block the capacity of CAFs to shape aggressive environments and significantly affect tumour progression.

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30. Biological characterization of novel chromeno[2,3-d]pyrimidinone as therapeutic agents for treatment of triple-negative breast cancer

Authors and Affiliations

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Abstract

Breast cancer is the most diagnosed cancer-type and the principal cause of cancer-related mortality in women, from which the triple-negative breast cancer (TNBC) subtype is the most lethal. In general, TNBC treatment options include conventional chemotherapy, radiation and/or surgery, but patient response is poor, leading to low 5-year survival rates. Thus, there is an urgent need for new therapeutic approaches, that overcome current clinical challenges of this disease. Considering that chromene-based scaffolds have proven to be attractive candidates for cancer therapy, our research group developed a series of new chromene[2,3-d]pyrimidinones and studied their anticancer potential against TNBC cells and safety profile.

A cell viability screening in several breast cancer cell lines allowed to identify two promising hit compounds with IC50 values in the low micromolar range, for TNBC cells. In the study of their mechanism of action, these chromenes showed powerful anticancer properties against TNBC cells, through the inhibition of cell proliferation, induction of cell cycle arrest and by triggering cell death through apoptosis.

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Additionally, using the ex vivo Chick Chorioallantoic Membrane (CAM) model, a single-dose treatment with each of the two chromenes induced significant tumor regression. Importantly, in vivo toxicity studies of these hit compounds revealed a safe profile in invertebrate (Caenorhabditis elegans) and vertebrate animal (wild-type C57BL6/J mice) models.

In conclusion, the two compounds identified in this study are promising drug candidates for TNBC treatment and valuable hits for future lead optimization, using the versatile synthetic platform that was developed in-house.

31. Effects of lactate transport inhibition by AZD3965 in muscle-invasive urothelial bladder cancer

Authors and Affiliations

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Abstract

Urothelial bladder cancer (UBC) is the 10th most common type of malignancy worldwide, being characterized by high levels of morbidity and mortality. Glucose avidity and high rates of lactate production by malignant cells characterize the metabolic switch from oxidative phosphorylation to accelerated glycolysis, known as the Warburg Effect. Increased extracellular lactate levels have been associated to multiple tumorigenic features, namely therapy resistance. To avoid cellular acidosis by internal lactate accumulation, monocarboxylate transporters (MCTs) are widely overexpressed in cancers and linked to aggressiveness, making them interesting targets for therapy. AZD3965, developed by Astrazeneca, is a MCT1-specific inhibitor that has been studied in different types of cancer, with promising results.

This work represents, the first pre-clinical evaluation of AZD3965 in bladder cancer, studying the effects in muscle-invasive UBC cell lines regarding distinct aggressiveness parameters. Additionally, we performed in vivo testing using the chick chorioallantoic membrane model assay, and studied several functional effects upon AZD3965 combination with cisplatin, the most commonly used chemotherapeutic agent in high-grade UBC treatment.

Our data revealed that AZD3965 has anticancer properties on UBC treatment that seem to be directly linked to MCT4 expression, as intense therapy resistance was observed under high expression of the biomarker. This compound compromised cell viability, proliferation and migration properties, and reduced tumor growth in vivo. When combining therapies, an apparent sensibilization to cisplatin was associated to low MCT4 expression. Despite these promising results, further testing is required to validate the effect of AZD3965 in UBC.

32. Antibody blockade of PSGL-1, a novel immune checkpoint protein, enhances T cell activation against B-cell lymphoma

Authors and Affiliations

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Abstract

Introduction: Most lymphoma subtypes are refractory to PD-1 and other immune checkpoint therapies. Since P-selectin glycoprotein ligand-1 (PSGL-1) was found to be an immune checkpoint protein promoting T cell exhaustion in mouse melanoma, we aimed to evaluate the PSGL-1 potential to stimulate T cell responses against B-cell lymphoma.

Materials and Methods: Human healthy donor T cells were cultured with irradiated Raji lymphoma B-cell line. T-cell activation was assessed by flow cytometry detection of CD25, CD69 and ELISA detection of IL-2 and IFNγ production. For in vivo studies, BALB/c mice were s.c. injected with mouse B-cell lymphoma cell line A20.

Results: We found that upregulation of CD69 and CD25 and IL-2 production in pre-activated T cells upon coculture with Raji cells was boosted by the PL1 human PSGL-1 mAb. Furthermore, PL1 increased the percentage of CD4+CD69+ T cells and IFN-γ and IL-2 production after coculture of in vitro exhausted-like T cells with Raji cells. In addition, PL1 increased the percentage of CD69+ and CD25+ activated cells after coculture of previously Raji-primed T cells with Raji cells. Upon autologous coculture of lymphoma and T cells from patients, we found that PL1 treatment enhanced T-cell activation (CD69+) in two patient samples.

Finally, treatment of A20 lymphoma-bearing mice with 4RA10 mouse PSGL-1 mAb reduced tumor growth and increased the percentage of tumor immune infiltrates. 4RA10-treated tumors had specific increased percentage of activated CD8+ tumor infiltrated T cells and a reduction of Treg populations.

Conclusions: We demonstrate for the first time that PSGL-1 antibody blockade enhances human T cell activation against lymphoma cells. Furthermore, we observed that anti-PSGL-1 treatment increased immune infiltration and reduced B-cell lymphoma growth. These findings support the notion that PSGL-1 can be a target for future immunotherapeutic options.

33. Transgenic TCR OT-I expression leads to T-cell acute leukemia development

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Abstract

T cell acute lymphoblastic leukemia (T-ALL) is a malignant disorder characterized by the proliferation and dissemination of aberrant immature T lymphocytes. T lymphocytes express at their surface the T cell receptor (TCR), which is essential during normal T cell development and function. A fraction of T-ALL cases express TCR at the leukemic cells surface, hence it is important to understand its role in a malignant context.

The goal of this study was to understand the role of TCR expression in leukemia development, as well as the impact of its stimulation in leukemic cells. A cohort of mice expression transgenic TCR OT-I, responsive to the SIINFEKL ovalbumin peptide (OVA), were followed for disease development. Immunophenotypic analysis of the leukemic cells was performed by flow cytometry. Leukemic and non-leukemic OT-I T cells were stimulated in vitro with OVA and their activation was evaluated by CD69 induction. To verify their malignant nature, OT-I leukemic cells were inoculated in Rag2-/- mice by intravenous injection.

Over 50% of TCR OT-I mice developed leukemia characterized by thymic lymphoma, splenomegaly, lymphadenopathy and dissemination to non-lymphoid organs. Leukemic cells expressed high levels of CD90, a T lymphocyte marker, demonstrating that these mice develop T cell leukemia. Surface expression of CD4, CD8, CD24, CD5 and TCR was also detected. When OT-I leukemic cells were inoculated in recipient mice, they rapidly develop fatal leukemia with the same molecular features. Surprisingly, when treated with OVA in vitro, OT-I leukemic cells had a poor induction of CD69, when compared to healthy OT-I lymphocytes, despite normal levels of surface TCR.

These results demonstrate that expression of the transgenic TCR OT-I leads to T cell leukemia development, but the TCR becomes poorly responsive to in vitro stimulation. Further investigation is needed to understand which gene mutations cooperate with transgenic TCR to develop leukemia and to assess whether the leukemic cell OT-I TCR responds in vivo to OVA stimulation.

34. Characterization of breast cancer immunity

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Abstract

Breast cancer (BC) may affect any woman in the world, who is past puberty. Aberrant expression of focal adhesion kinase (FAK) has been identified in BC tissues. FAK is involved in main tumor signaling pathways. Moreover, FAK contribution for metastasis has been reported to be extremely relevant, by reducing cell-cell adhesion, improving the attachment to endothelial cells and regulating the new tumor microenvironment (TME) for sustaining the tumor adaptation to a secondary site. Myeloid-derived suppressor cells (MDSC) promote an immunosuppressive microenvironment in BC, facilitating immune evasion by cancer cells and enabling their thriving.

We hypothesized that the inhibition of FAK in BC may modulate the TME toward a more permeable environment that allow an efficient infiltration of effector immune cells triggered by vaccination, while the synergy with the inhibitor MDSC may overcome immunosuppression resulting in a strong anti-tumor immunity.

Female C57BL/6J mice were inoculate with E0771 BC cells. TME cells were used to create 3D spheroids that were treated with FAK and/or MDSC inhibitors, and co-cultured with splenocytes from immunized mice, at different treatment schedules. To assess the best anti-tumor effect the spheroid diameter, invasion and immune infiltration were evaluated.

The synergy between the MDSC inhibitor followed by simultaneous administrations of the FAK inhibitor and splenocytes led to the most promising outcomes by impacting the 3D spheroid growth and sprouting. The simultaneous use of both inhibitors and splenocytes, also showed a promising anti-tumor effect.

35. The Golgi ion channel TMBIM4 controls cancer cell invasion and survival by reprograming the transsulfuration pathway

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Abstract

Several Golgi functions are associated with events linked to cancer progression. Despite the role of the Golgi in protein and lipid modification and trafficking, less explored aspects of this organelle can contribute to cancer cell fate by affecting signal transduction, ion homeostasis or metabolism. This work aims at exploring the impact of the Golgi cation channel Transmembrane BAX Inhibitor Motif Containing 4 protein (TMBIM4) on cancer cell survival and motility.

To explore the impact of TMBIM4 on cancer cell survival, TMBIM4 or a TMBIM4 null mutant were overexpressed in U2-OS cells. To simulate a tumour environment with limited nutrients and pro-growth signals, cells were cultured for long periods without media replacement. Under these conditions, cells overexpressing TMBIM4 remained alive while control cells did not. This effect was pH-dependent as TMBIM4 only promoted cell survival at pH 7.4-7.8, but not at more acidic pHs. After prolonged periods of cultivation, TMBIM4 increased the expression of key transsulfuration enzymes and protected cells from lipid peroxidation and DNA damage. The addition of cysteine or GSH-EE to the media contributed to the long-term survival of control cells. Thus, suggesting that the transsulfuration pathway is relevant for TMBIM4-induced cell survival, possibly by providing cysteine for protection against oxidative stress.

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Gene expression analysis of tumours (TCGA) revealed that TMBIM4 is expressed at high levels in gliomas and is positively correlated with glioma grade and with a reduction in low-grade glioma patient survival rate. The KD of TMBIM4 limited in vitro cancer cell invasion and in vivo tumour growth in a mouse orthotopic glioma model.

Data obtained so far support further exploration of TMBIM4 as a potential cancer progression biomarker, and as a new therapeutic target.

36. Impact of G9a/DNMT inhibition in combination with immunotherapy behind a sustained anti-tumoral response in bladder cancer upon rechallenge

Authors and Affiliations

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Abstract

Introduction: Epigenetic modifications are pivotal in driving the development and progression of bladder cancer (BC), influencing the tumor microenvironment. A G9a/DNMT inhibitor, CM-272, was shown to have anti-tumor effects in combination with immune checkpoint blockade in a BC mouse model, with no significant tumor reappearance after completing the combination treatment. Hence, the aim of this study is to discover the mechanisms underlying the prolonged anti-tumoral effects observed with CM-272 in combination with anti-PD-L1.

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Materials and Methods: Using an immunocompetent syngeneic mouse model of metastatic BC (Pten F/F; Trp53 F/F, DKO), 4K5 cells, previously established from a mouse bladder tumor (Pten -/-; Trp53 -/-), were injected subcutaneously and treatment with a combination of CM-272 and anti-PD-L1 was applied (1st challenge). Next, tumors were surgically removed, and after recovery, mice were rechallenged with either 4K5 or a distinct syngeneic mouse BC cell line originating from the same DKO strain (3K20) (2nd challenge). All tumors were harvested for histological and flow cytometry analyses.

Results: Upon rechallenge, mice inoculated with the same cell line as in the 1st tumor challenge did not display tumor growth in any of the groups, indicating the generation of immunity against these cells. In contrast, the growth of 3K20 tumor cells was prevented preferentially in animals previously treated with the combination, indicating an enhanced immune response upon treatment. In the rechallenged mice, we observed that tumors collected from mice previously treated with CM-272 and anti-PD-L1 exhibited higher infiltration of CD8+ T cells and M1 macrophages than control mice.

Conclusions: The increased tumors' elimination upon rechallenge in CM-272 and anti-PD-L1 pre-treated mice underscores an enhanced immune response against distinct BC cell lines post-treatment. Next, we intend to explore the immune memory compartment, unraveling key immune mechanisms behind these findings.

37. Design and development of a strategy to obtain a specific domain of the E3 ubiquitin-ligase protein Hakai

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Abstract

Introduction: Hakai is a E3 ubiquitin ligase that mediates ubiquitination of E-cadherin leading to its degradation, which in turns causes downregulation of cell-to-cell contacts and induces epithelial-mesenchymal transition (EMT). The HYB domain was first described in Hakai as an atypical phosphotyrosine binding domain. The HYB domain is required for the specific interaction of Hakai with E-cadherin, in a phosphotyrosine dependent manner. Therefore, Hakai, and specifically its HYB domain, is a promising therapeutic target for novel anticancer strategies. In this study we aim to clone and purify HYB domain of Hakai.

Methods: Gateway technology were used to clone the HYB domain sequence into pDest-565 expression vector by two sequential recombination reactions based on the recognition of modified att sequences. This vector contains a GST tag and a PreScission Protease cleavage site that allows the tag to be cleaved and separated using a PreScission Protease cleavage column.

Results: The HYB domain sequence of Hakai was cloned into pDest-565 vector and expressed as GST-fusion protein in E.coli BL21(DE3) cells by a IPTG inducible system. The GST tag will allow us to purify the expressed HYB fragment with affinity chromatography columns. An even higher degree of purification can be achieved with a subsequent molecular exclusion chromatography.

Conclusions: In summary, we have shown the cloning of the sequence encoding the HYB domain that will help to further explore the potential of the HYB domain as a therapeutic target in cancer by providing a model to test the interaction with specific Hakai substrates.

38. Cannabinoids and ERα: how hormones can affect their relation in breast cancer?

Authors and Affiliations

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Abstract

Introduction: The development of resistance is still one of the main challenges regarding breast cancer (BC) therapy. Because of that, novel therapeutic approaches are in constant development and application. Regarding this, our group has already demonstrated the promising anti-tumor effects of cannabinoids, including cannabidiol (CBD), in estrogen receptor-positive (ER+) BC, through the modulation of specific targets such as aromatase, ERα and androgen receptor (AR). Here, our aim was to evaluate the influence of cannabinoids on ERα and how tumor microenvironment affects its activity.

Methods: For CBD and other minor cannabinoids, cannabigerol (CBG), cannabidivarin (CBDV), cannabinol (CBN) and cannabichromene (CBC), their dependence on ERα was evaluated on MCF-7aro cells. VM7Luc4E2 cells were used to access the agonistic/antagonistic activity exerted by each cannabinoid in the presence of testosterone (T) or estradiol (E2).

Results: Our results indicated that all the cannabinoids exert effects on MCF-7aro cell viability in an ERα-dependent manner. Moreover, we verified that CBD, CBN and CBC act as ERα antagonists, while CBDV acts as an agonist and CBG has no effects. In the presence of T, these effects were maintained, while in the presence of E2 all the compounds lost their effects on ERα activity. Corroborating these results, the cannabinoids with antagonistic activities compromised the transcription of ERα-target genes and reduced ERα protein levels.

Conclusions: This work shows for the first time that not only CBD, but also some minor cannabinoids exert important effects on ERα, one of the main therapeutic targets in ER+ BC, showing that they might be particularly appealing as an adjuvant therapeutic approach. Thus, this work reinforces the potential of cannabinoids in BC treatment and expands the knowledge regarding minor cannabinoids.

39. Generation of ex-vivo 3D organoid culture from surgically-derived tissue of paediatric brain tumours

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Abstract

Despite significant improvements in cancer care and diagnosis, paediatric brain tumours (PBTs) remain a leading cause of death in children. The current multimodal approach to these rare tumours has led to enhanced survival rates, but it is also accompanied by notable long-term sequelae. As part of the nationwide PRECISEKIDS project, our multidisciplinary team actively contributes to the molecular characterisation of PBT cases, submitting paediatric solid tumours, including PBTs, to NGS studies to provide clinicians with crucial molecular insights for each case. While we are having remarkable advances in understanding the molecular biology behind these diverse tumours, the translation of all this knowledge into target therapies remains a challenge. Thus, our main aim is to generate patient-derived organoid (PDO) cultures from surgically derived tumour tissue of PBTs. Once established and molecularly characterised, these PDOs can be submitted to drug screening assays and constitute an important molecular tool capable of guiding precision medicine in the paediatric cancer context. Our key tasks involve collecting surgical material from PBTs to generate PDOs and conducting genomic characterisation of both PBTs and their corresponding PDOs using an NGS panel dedicated to paediatric cancer. In collaboration with Centro Hospitalar Universitário São João, we have collected surgical material from twenty PBTs which resulted in the successful establishment of thirteen PDO cultures from pilocytic astrocytomas, a pleomorphic xanthoastrocytoma, an ependymoma, a medulloblastoma and high-grade diffuse gliomas. The organoids started to emerge in culture 8-15 days after seeding, and immunofluorescence analysis using confocal high-content microscopy imaging with glial fibrillary acidic protein as a marker demonstrated glial differentiation in all the established cultures.

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Furthermore, NGS analysis confirmed that the generated PDOs could maintain the genomic profile of their corresponding primary tumour for over a month, underscoring the potential of PDOs for a personalised medical approach for PBTs. Our next steps involve performing RNAseq in PDO cultures to complete the molecular characterisation of these cultures. Moreover, we have initiated the process of optimising the protocol for conducting drug screening assays in the generated PDOs.

40. Targeting glutamine metabolism on tumor micro-environment using a pharmacological approach

Authors and Affiliations

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Abstract

Introduction: Targeting glutamine metabolism with pharmacological approaches such as 6-diazo-5-oxo-L-norleucine (DON) has been proposed as a promising anti-tumor strategy, owing to cancer cell reliance on glutamine driven by oncogene like Myc. In this study, we investigated in vivo efficacy of a DON prodrug, JHU083, which selectively activates in tumors with enriched proteases, on a genetically engineered murine model of endometrioid cancer (APPC). To uncover key changes implicated in anti-cancer efficacy and tumor microenvironment restructuring, we performed scRNAseq on APPC tumors.

Materials and Methods: JHU083 or vehicle control was administrated to APPC mice by intraperitoneal injection for three weeks. At the end point, endometrioid tumors were harvested for scRNAseq using the 10X Genomics Chromium platform. Cell cluster annotation, differential gene expression and statistical analyses were performed by Dr. Alex Lemenze at Rutgers University.

Results: scRNA-seq performed revealed 22 cell clusters in the murine endometrioid tumors. JHU083 treatment increased proportions of endothelial cells, mesothelial cells, and a subset of fibroblasts (fibroblast 4) ($p<0.005$). Conversely, JHU083 reduced fibroblast 5 cluster proportion ($p<0.005$), and modestly reduces proportions of two macrophages sub-clusters ($p=0.05-0.06$). To verify the above endothelial cell results, we performed immunohistochemistry using an anti-CD31 antibody and observed an increased blood vessel density in tumors of JHU083-treated mice. This result may explain reduced hypoxia reported previously and implicate normalized and/or increased blood/lymphatic flow in JHU083-treated tumors.

Conclusion: The scRNAseq results demonstrated significant tissue remodeling upon glutamine inhibitors treatment, which may contribute to the concurrently reduced tumor stage and tumor burden. Further investigation will focus on the "on target" cell populations with metabolic expression shifts known to be regulated by glutamine inhibition.

41. Epigenetics as a source of predictive biomarkers for immunotherapy outcome in clear cell renal cell carcinoma

Authors and Affiliations

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Abstract

Introduction: Immunotherapy based on immune checkpoint inhibitors (ICI) results in durable responses in a subset of patients with advanced clear cell renal cell carcinoma (ccRCC), but mechanisms driving resistance are poorly understood. Therefore, the identification of predictive biomarkers is still an unmet need. Recently, a close relation between DNA methylation and the immunogenic status of the tumor microenvironment has been proposed for several solid tumors. In this work we evaluated the epigenetic status of ccRCC before immunotherapy to disclose the possible role of DNA methylation in ICI response, and to identify methylation signatures that predict ICI outcome.

Materials and Methods: We included 16 advanced ccRCC patients treated with nivolumab-ipilimumab (anti-PD1/anti-CTLA4) at first line. DNA was extracted from FFPE tumor biopsies. DNA methylation was quantified with the MethylationEPICv2 array, which measures the methylation levels of 936,990 CpG sites across the genome. Data was annotated to the hg38 genome and bioinformatics was performed with R/Bioconductor.

Results: Patients with radiological response (n=9) showed longer progression-free survival (PFS) and overall survival (OS) than non-responder patients (n=7). Differentially methylation patterns were identified, with a signature of 256 CpG sites that predicted a 6-months response to anti-PD1/anti-CTLA4. Also, we reported a methylation profile associated with longer PFS. Differentially methylated CpG sites were enriched in pathways that modulate immune response such as TNF signaling, PD-L1/PD-1 checkpoint, IL-17 signaling, and Th17 differentiation. We also found aberrant methylation of the PI3K-Akt signaling pathway, which regulates immune checkpoints and the sensitivity to ICI.

Conclusions: Our results confirm that DNA methylation plays a role in the response to ICI in advanced ccRCC. These immune-related methylation profiles can predict response to anti-PD1 therapy, representing a source of predictive biomarkers for ICI outcome.

42. Insights into the impact of epigenetic drugs in lung cancer

Authors and Affiliations

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Abstract

In spite of recent advances in lung cancer treatment based on personalized medicine and immunotherapy based on checkpoint inhibitors, lung cancer still represents the second most frequently diagnosed tumor and the leading cause of death due to cancer. Clinical efficacy varies between patients and most strategies fail to induce long-lasting efficient cytotoxic responses. Thus, new combined therapies are an urgent need for the most mortal cancer. Given the extensive alterations in the methylomes of human cancer and their likely role in therapy response, it is not surprising that new epigenetic therapies are being considered

Here, we investigated the impact of different epigenetic drugs on two lung adenocarcinoma cell lines NCI-H2009 (stage 4) and NCI-H2087 (stage 1). We have selected epigenetic agents already approval for hematological cancer: AZA (5- Azacytidine), a specific and potent inhibitor of DNMT1, and two pan-HDAC inhibitors: Panobinostat and Vorionostat. We have used a XTT proliferation kit to determine their sensitivity and corresponding IC50 values. The expression of several Epithelial-mesenchymal transition (EMT) genes was measured by real-time PCR (qPCR) in treated vs. untreated cells. We also evaluated cell cycle by flow cytometry.

Cell viability measurements did not reach IC50 until 48 and 72 hours of treatment. Both cell lines were highly resistant to AZA, followed by Vorionostat and showing high sensitivity to Panobinostat. During the treatment especially with AZA, cells behavior in an intriguing manner, loosing their contacts and forming aggregates as becoming more mesenchymal and less epithelial. The qPCR results confirmed our suspicions suggesting a dedifferentiation from an epithelial state to a mesenchymal-like state during the epigenetic treatment.

Although further confirmation is needed, our results indicate that epigenetic treatment could promote a transition towards a more invasive mesenchymal phenotype in lung cancer.

43. Deciphering novel mechanisms of WNT6-driven chemotherapy resistance in human glioblastoma

Authors and Affiliations

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Abstract

Introduction: Glioblastoma (GBM) is among the deadliest cancers, for which treatment remains ineffective. Thus, it is urgent to identify new biomarkers predictive of therapy response, and elucidate their underlying mechanisms, ultimately uncovering novel therapeutic opportunities. Our team showed that WNT6, a WNT pathway activator, is a GBM oncogene, which could also potentiate chemoresistance. Thus, further studies are required to explore this putative role of WNT6 in therapy resistance, while also unravelling its underlying molecular mechanisms.

Materials and Methods: GBM cell lines U373 and U87 were genetically engineered to express differential levels of WNT6, using knockdown and overexpression approaches, respectively. In vitro, after identifying the TMZ half maximal inhibitory concentration (IC50), its effect on GBM cells' aggressiveness features was analysed, including on cell viability, proliferation, and colony formation. In vivo, NSG mice were intracranially injected with WNT6-high/low U87 cells and posteriorly treated with TMZ or vehicle. The overall survival of the different groups of mice was determined and compared. Additionally, RNA-sequencing data was generated from these cell models to identify putative molecular mediators that could underlie WNT6-driven TMZ resistance.

Results: In vitro U373 WNT6-low cells presented decreased TMZ IC50, as well as, reduced cell viability, colony formation capacity and cell proliferation. Importantly, similar findings were also validated in the WNT6 overexpression model. In vivo, mice with WNT6-high tumors subjected to TMZ showed a tendency for decreased overall-survival in comparison to WNT6-low, although not reaching statistical significance. RNA-Sequencing experiments identified targets, such as the PI3K-Akt pathway, which may explain the observed in vitro and in vivo effects.

Conclusion: WNT6 could be negatively influencing GBM's response to standard-of-care treatment with TMZ, and its modulation might improve treatment effects.

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44. Colon cancer patients with MSI tumors enriched in CCL18/macrophages have worse overall survival

Authors and Affiliations

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Abstract

Recently we identified the chemokine CCL18 as a major pro-invasive factor involved in macrophage-mediated colon cancer (CC) cell invasion, produced when macrophages differentiated within CC patients-derived decellularized matrices (1). We also demonstrated that CCL18 expression is highly increased at the invasive front of human CC tumors. In this study, the expression levels of CCL18, its putative receptor CCR8 and macrophage markers (CD68, CD80 and CD163) and their correlation with the clinicopathological characteristics and prognosis in patients with CC were analyzed by TCGA RNA sequencing data. Cox proportional hazard regression model was performed to assess the association between CCL18/macrophage-associated markers and CCL18/CCR8 expression and overall survival (OS) in patients with CC.

As a consequence, we found that the expression of CCL18 was markedly elevated in CC samples as compared with the adjacent normal tissues and acted as an independent prognostic factor of OS in patients with tumors characterized by microsatellite instability (MSI). Subsequently, Pearson correlation analysis revealed that CCL18 had a positive correlation with CCR8, CD86, CD80 and CD163 expression in CC samples. CCR8 expression was also up-regulated in CC tissues and was associated with poor survival in patients with MSI tumors.

Taken together, our findings demonstrated that the dysregulation of CCL18/CCR8 axis and its association with the presence of macrophages could predict poor prognosis in CC-patients with MSI tumors.

1) Pinto ML et al., Biomaterials. 2017 doi: 10.1016/j.biomaterials.2017.02.004

45. Radiocommunication: Unraveling the role of extracelullar vesicles in rectal cancer radioresistance

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Abstract

Rectal cancer (RC) is responsible for over 30% of the colorectal cancer burden worldwide. Neo-adjuvant radiotherapy (RT) is the standard treatment method for RC, but still leaves over 30% of the patients experiencing no response or local recurrence after treatment due to variable levels of radioresistance. As such, there is an urgent need for the development of more efficient therapies, to be combined with RT. We postulate that radioresistance may be enhanced by intercellular communication within the tumour and as such, we set out to uncover radioresistance molecular players enclosed in extracellular vesicles (EVs) from RC cells.

To effectively study RC radiobiology, we developed and characterized a 3D rectal cancer model – the multicellular spheroid, according to MISpheroID proposed guidelines, and applied the standard short RT scheme used in RC patients. This system was upscaled to isolate EVs via density gradient UC coupled with SEC. Isolation was quality controlled via NTA, Western Blotting and TEM. EV isolates were submitted to proteomic analysis and RNAseq.

RC spheroids were produced with two cell lines, which vary in size and compactness - SW837 cell line forms smaller, more compact spheroids, when compared to SW1463 cell line. SW837 spheroids were used to collect EVs, and RT on these spheroids provokes alterations in the EV proteome – RT-EVs contain proteins related to nucleotide metabolism and DNA repair, but lose proteins related to epithelial adhesion, signal transduction, and anti-tumour immune response.

We have successfully developed two RC 3D models suitable for radiobiology studies and EV isolation. RT influences the proteome of RC EVs. We aim to characterize EVs from SW1463 spheroids in order to detect differences in the EV proteome that explain radioresistance. We will also characterize these EVs via RNAseq. Finally, we aim to validate our findings in human samples, by exploring radioresistance markers in circulating EVs as well as understanding how they functionally contribute to radioresistance.

46. EZH2 inhibition leads to increased NK cell-mediated killing of prostate cancer cells

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Abstract

Although prostate cancer (PCa) is one of the most incident malignancies, it currently lacks treatment options for advanced forms of disease. EZH2, responsible for the trimethylation of histone 3 at lysine 27 (H3K27me3), which leads to transcriptional repression, has been associated with PCa progression. Natural killer (NK) cells target tumor cells through an array of activating and inhibitory receptors that interact with cell surface ligands on target cells. Previous studies suggest a role for EZH2 in the impairment of immune responses, however, its impact on PCa evasion mechanisms to NK cell-mediated immunity remains unknown.

We aimed to increase prostate cancer immunogenicity through treatment with CPI- 1205, a novel EZH2 inhibitor, enhancing NK cell recognition.

EZH2 and H3K27me3 expression was assessed in PCa patient tissues by immunohistochemistry. PCa cells were treated with CPI-1205, and expression of NK cell ligands was evaluated by RT-qPCR and flow cytometry. NK cells were isolated and co-cultured with CPI-1205-treated prostate cells, and NK cell cytotoxicity assays were performed.

EZH2 and H3K27me3 displayed increased expression with disease progression. Treatment of PCa cells with CPI-1205 resulted in the upregulation of NK cell activating ligands, at transcriptional and cell surface levels, particularly ULBP2/6. Furthermore, NK cell-mediated killing of PC-3 cells (a PCa bone metastatic cell line) was increased upon EZH2 inhibition.

Our findings suggest that EZH2 inhibition with CPI-1205 can modulate PCa immunogenicity, rendering PCa tumors more sensitive to NK cell-mediated killing, supporting the design of novel epigenetic-based therapeutic approaches for PCa patients.

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47. ETV6::JAK2 fusion promotes central nervous system invasion in B-cell acute lymphoblastic leukemia

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Abstract

Acute lymphoblastic leukemia (ALL) is the most frequent pediatric malignancy and remains an important cause of mortality in both children and adults. A subgroup of B-cell precursor ALL (BCP-ALL) patients carries specific genetic alterations, including JAK2 fusions, such as ETV6::JAK2, and CDKN2A deletions, and have bad prognosis. Disease relapse in these patients is often associated with central nervous system (CNS) infiltration. Here, we hypothesized that ETV6::JAK2 fusion is a risk factor for developing aggressive CNS-infiltrating BCP-ALL.

To study the impact of JAK2 signaling in CNS involvement by B-ALL, we generated ETV6::JAK2;Rag2-/-;Cdkn2a-/- mice, which express a constitutively active ETV6::JAK2 fusion kinase in the lymphoid lineage, in a deficient Rag2 and Cdkn2a background.

Indeed, ETV6::JAK2;Rag2-/-;Cdkn2a-/- mice exhibited early-onset B-ALL development with more frequent CNS invasion (e.g., paraparesis/paraplegia), in comparison to Rag2-/-;Cdkn2a-/- littermates. Immunohistochemistry analyses of diseased ETV6::JAK2;Rag2-/-;Cdkn2a-/- and Rag2-/-;Cdkn2a-/- mice revealed leptomeningeal invasion by p-STAT5+B220+PAX5+ cells. In vitro treatment of primary leukemic cells with a JAK2 inhibitor (AZD1480) impaired significantly ETV6::JAK2;Rag2-/-;Cdkn2a-/- cells survival comparing to Rag2-/-;Cdkn2a-/- cells. To assess the dynamics of CNS invasion by B-ALL, we transplanted CD45.2-expressing ETV6::JAK2;Rag2-/-;Cdkn2a-/- and Rag2-/-;Cdkn2a-/- cells into Rag2-/-;CD45.1+ recipients and monitor for disease development by detecting leukemic cells in the cerebrospinal fluid (CSF) at different timepoints, via flow cytometry analysis. Ten days post-injection, despite the presence of leukemia in peripheral blood in both groups, we only detected CD45.2+B220+ cells in CSF of recipients injected with ETV6::JAK2;Rag2-/-;Cdkn2a-/- cells.

In conclusion, ETV6::JAK2 fusion promotes leukemia survival, accelerates disease development, and confers higher neurotropism to leukemic cells.

48. Proteomic profiling of prostate cancer extracellular vesicles: a comparative study between primary and bone-metastasis murine cell lines

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Abstract

Introduction: Prostate cancer (PCa) is a global health concern, namely when the disease is spread to the bone, increasing patients' morbidity and mortality. Tumor-derived extracellular vesicles (EVs) have emerged as key players in the progression of cancer. The content of EVs is intricately linked to the characteristics of the originating cells. To elucidate the influence of EVs in PCa progression, we characterized EVs derived both from primary and bone metastasis (BM)-derived murine cell lines.

Methods: We utilized a luciferase-mCherry tagged CP2 cell line derived from a C57BL/6 Ctnnb1pc(ex3) Δ/+Ptenpc+/- mouse to induce BM, subsequently establishing the BM-derived CP2-Bo2 cell line. EVs were isolated via ultracentrifugation with sucrose cushion, characterized using Nanoparticle Tracking Analysis and BCA assay, and subjected to Liquid Chromatography-Mass Spectrometry. Differential expression, STRING, KEGG pathway, and Gene Ontology (GO) analyses were conducted.

Results: Our findings revealed that CP2-Bo2-derived EVs exhibited differential expression of proteins involved in focal adhesion, metabolism, migration, proliferation, and cell survival. Moreover, GO analysis also revealed their association with bone development processes.

Conclusion: The distinct protein expression patterns identified in EVs derived from BM cells suggest that these vesicles participate in key aspects of cancer progression. Furthermore, the association of these EVs with bone development indicates a likely mechanism by which PCa cells interact with the bone microenvironment. Our research underscores the significance of EVs in PCa progression, particularly in the context of bone metastasis.

49. Role of Hakai in colon cancer stem cell

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Abstract

Introduction: Carcinoma, the most common type of cancer, arises from the malignant transformation of epithelial cells. At the early stages of carcinoma progression, epithelial cells can undergo a program known as epithelial-mesenchymal transition (EMT). EMT is characterized by the loss of E-cadherin, a tumour suppressor responsible for cell adhesion in the epithelium. The E3 ubiquitin-ligase Hakai was the first reported posttranslational regulator of the E-cadherin stability. Hakai is involved not only in the control of cell-cell contacts, but also in the control of cell proliferation, cell-substrate adhesion and invasiveness. Taking into consideration the strong evidence that supports the link between the EMT process and the development of cancer stem cell (CSCs) we aim to study of the involvement of Hakai in the CSC properties.

Materials and Methods: We used an inducible system of HT29 colon cancer cells using a viral transduction of shRNA-Hakai in CSC conditions to induce the formation of tumorspheres. Hakai-silencing increases colorectal tumorsphere size and regulate stem cell markers. Moreover, by proteomic analyses using Trapped Ion Mobility Spectrometry time-of-flight (timsTOF) technology, we studied the effect of Hakai-silencing tumorospheres cultivation in self-renewal capability of CSCs and found potential novel cancer related proteins involved in CSC.

Results: We have identified LRP4 as a new potential Hakai-interacting protein. LRP4 is a member of the Lipoprotein receptor-related protein family and is proposed as a regulator of Wnt signalling pathway. Hakai overexpression reduces LRP4 protein levels, induces its ubiquitination and consequently degradation via proteasome.

Conclusions: These data suggest that Hakai could impact on Wnt cascade, a critical regulator of cancer stem cells.

50. Exploring the role of mutant KRAS in mediating the crosstalk between colorectal cancer cells and fibroblasts: implications for invasion and metastatic potential

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Abstract

Oncogenic KRAS signaling has proved to exert numerous effects in the orchestration of the tumor microenvironment (TME). The recognition of the importance of the TME in disease progression and therapy response has changed the view of cancer therapy, and highlighted novel therapeutic targets involved in the interplay between cancer cells and their microenvironment. Within the TME, cancer-associated fibroblasts stand out as prominent stromal constituents, acknowledged for their capability to drive the invasive behavior of colorectal cancer (CRC) cells. Therefore, in this work, we aimed to explore and characterize the role of mutant KRAS in mediating CRC cells-fibroblasts crosstalk.

By challenging KRAS mutated cells with fibroblast-conditioned media, we observed that KRAS silencing decreased the invasive capacity of the cancer cells. Analysis of the conditioned media revealed the presence of high levels of HGF. Neutralization and supplementation experiments showed that HGF induced invasion in a KRAS-dependent manner. Consequently, we observed that KRAS regulates the expression of the HGF receptor, C-MET and that its silencing decreases the levels of fibroblast-induced invasion, suggesting a role of the HGF-C-MET axis in regulating the invasive properties of these cells.

Our data bestows fibroblasts a pivotal role in mediating KRAS oncogenic effects, particularly in facilitating invasion. It provides valuable insights into the mechanisms underlying several clinical observations. For instance, we are presently devoted to exploring the possible connection between the invasive potential facilitated by the interaction between KRAS and fibroblasts and the peritoneal dissemination commonly associated with mutant KRAS consensus subtype 4 CRC. Additionally, we aim to shed light on the mechanisms underlying the dismal prognosis of mutant KRAS CRC liver metastasis and the propensity for lung recurrence following resection of the liver metastasis.

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51. A novel thiazole inhibitor of Lysyl Oxidase Like 2 alters breast cancer cell migration

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Abstract

Lysyl oxidases (LOX) play a crucial role in facilitating the cross-linkage of elastin and collagen within the extracellular matrix. They have been associated with enhanced cancer cell migration and the development of metastases. LOX-like 2 (LOXL2) expression has been associated with a worst prognosis in breast cancer (BC), and can thus constitute a therapeutic target.

To identify new LOXL2 inhibitors (iLOX), docking studies were conducted, with compounds already described in the literature, to gain insights into possible binding modes to LOXL2. Based on the results obtained from these studies, indoline-aminoalkylphenol compounds available in house were tested. To assess their LOXL2 inhibitory activity, an Amplex Ultra Red technique was used. Cell viability was evaluated by the MTT assay in BC (MDA-MB-231) and normal-like (MCF10A) cell lines. The effect on the cell migration of cells was investigated using the wound healing assay, transwell assay for chemotaxis/chemoinvasion, and single cell tracking for random migration.

A thiazole compound derived from 2-(Indolin-1-yl(4-(trifluoromethyl)phenyl)methyl)-4-nitrophenol effectively inhibited LOXL2 with an IC50 value in the low micromolar range. This compound exhibited low cytotoxicity. At non-toxic concentrations, it did not interfere with collective cell migration and slightly increased the speed of individual migration. Importantly, it significantly reduced chemotaxis and chemoinvasion in MDA-MB-231 cells.

In this study, we have uncovered a novel LOXL2 inhibitor capable of altering the mobility of MDA-MB-231 cells. This inhibitor holds promise for further refinement, potentially paving the way for iLOX utilization as a treatment option for BC.

52. Development and Characterization of ovarian cancer zAvatars as a screening platform for ovarian cancer personalized therapy

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Abstract

Ovarian Cancer (OC) is the leading cause of death from gynecologic cancer, mostly due to the non-specific symptoms of the disease. OC is also characterized by different subtypes with differing clinicopathologic features and behaviors. Despite the high heterogeneity among patients, OC treatment is still based on the "one-size-fits-all" approach, and treatments may prove to be successful for some patients but not for others, especially in advanced stages of the disease.

To personalize treatment, our lab is developing OC patient-derived zebrafish xenografts (zAvatars) to be used as a screening platform for OC therapies. To achieve this, we are performing a co-clinical study to test the predictiveness of the model.

zAvatars were generated with patient tumor cells and treated exactly with the same therapy as their corresponding patient. Clinical responses of 22 patients were individually compared with their matching zAvatar-test. Smears were made to characterize the original tumor sample. Characterization of smears and zAvatars was performed by immunofluorescence confocal microscopy to assess different cell markers: PAX8, CA125, CD68, CD3, and α SMA. To assess drug sensitivity, cells positive for activated caspase 3 were quantified.

The comparison of the original tumor sample smear and the zAvatars revealed the maintenance of all cell markers in zAvatars. Different cell markers were expressed in very different proportions between patients, demonstrating the heterogeneity of OC.

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Regarding predictiveness in a total of 22 zAvatars, 20 matched the patient's clinical response, corresponding to an accuracy of 91%, with a negative predictive value (PV) of 100% and a positive PV of 87%.

The presence of the same cell markers in smears and matching zAvatars allows us to conclude that there is a good representation of the initial tumor sample. More importantly, the OC zAvatar shows an outstanding predictive value and therefore it can become a major breakthrough in patient management to optimize treatment options.

53. Differential protein and glycan packaging into extracellular vesicles in response to 3D gastric cancer cellular organization

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Abstract

Introduction: Alterations in the glycosylation machinery are common events associated with cancer progression that lead to the synthesis of aberrant glycan structures. These are involved in cancer hallmarks affecting patient prognosis and survival. Extracellular vesicles (EVs) shed by cancer cells carry several functional molecules, such as miRNAs, proteins, and glycans, holding modulatory capacity on the recipient cell to promote tumor progression. Our group has previously identified the presence of aberrant glycan structures in EVs released by cancer cell models. Current 3D cell culture methods have been shown to better mimic cancer in vivo phenotypes compared to monolayer cell culture. However, it remains to be clarified the effects of 3D cell culture conditions on the EV glycosylation molecular features. The aim of this study is to evaluate the glycosylation profile and the proteomic content of EVs derived from established glycoengineered cancer cell models by applying different cell culture methodologies, namely 3D and 2D cell culture.

Methods: EVs were isolated by Ultracentrifugation followed by size exclusion chromatography from 2D and 3D conditions were characterized, and their glycosylation profile was assessed using specific glycan-binding monoclonal antibodies, and lectins. Moreover, the EV proteome was analyzed using mass spectrometry.

Results: Our results revealed that 3D cellular architecture prompt cells to produce and release more EVs carrying distinct glycosignatures, including a differential packaging of proteins carrying STn glycan, branched and bisecting N-glycans, and the fucosylated glycans. Additionally, the protein signatures found in 3D-derived EVs also

showed a correlation with gastric cancer patients' clinical data

Conclusion: This study highlights the importance of cell culture methodologies when studying EVs and their glycosylation and protein content to properly assess their biological function and as sources of biomarkers to better mimic patient's data.

54. Characterization of the immune microenvironment of medulloblastoma's molecular subgroups

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Abstract

Introduction: Medulloblastoma is the most common pediatric malignant brain tumor and comprises four main molecular subgroups: WNT, SHH, non-WNT/non-SHH (Group 3, and Group 4). The immunological microenvironment of medulloblastoma is poorly understood, which hinders a rational immunotherapy approach. Recently, we reported the immune checkpoints profile of medulloblastomas, and found the absence of PD-1, PD-L1 or CTLA-4 expression, contrasting with higher levels of CD24, CD47, and B7-H3. Objectives: We aimed to characterize the immune microenvironment profile of medulloblastomas.

Methods: The mRNA of 88 human medulloblastomas was assessed through the PanCancer Immune Profiling Panel of NanoString, containing 730 immune-related genes. The expression levels comparison between molecular subgroups were analyzed using the R software. Further validation was performed in two public microarray datasets.

Results: Comparisons between subgroups, revealed that the WNT subgroup exhibits a lower immune infiltration with a higher abundance of myeloid cells, expressed high levels of ALCAM and TNFRSF11B, of the chemokines CCL2 and CXCR4, and of the anti-inflammatory cytokines TGFB1 and VEGFA. The SHH subgroup displayed a high abundance of infiltrated M2-like macrophages, and a significantly higher expression of TGFB2, CXCR4 and CXCL12. Groups 3 and 4 showed a similar profile, with both subgroups presenting a higher abundance of exhausted CD8 T cells, as well as a lower myeloid cell infiltration.

Conclusions: The immune microenvironment of medulloblastoma displays distinct profiles among molecular subgroups. In short, the SHH and WNT subgroups showed the presence of infiltration of anti-inflammatory myeloid cells, whereas groups 3 and 4 had evidence of immune exhaustion. This characterization could lead to new insights on the application of immunotherapeutic approaches in medulloblastoma.

55. A positive feedback loop between IFN-gamma signaling and cholesterol uptake sustains PD-L1 expression in breast cancer cells

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Abstract

Introduction: Cholesterol uptake via binding of the low-density lipoprotein (LDL) to the LDL receptor (LDLR) has been shown to induce aggressive behaviors in breast cancer (BC) cells, including increased proliferation, intravasation, and metastasis. The regulation of LDLR expression at the tumor microenvironment is, however mostly unknown.

Materials and Methods: Here we tested a small panel of cytokines, known to be present in the tumor microenvironment, for the capacity to induce LDLR expression and cholesterol uptake in (BC) cells.

Results: We show that IFN-gamma (IFNg) induces LDLR protein expression in three different BC cell lines in a JAK-STAT-1-dependent way. Moreover, treatment with IFNg increases LDL uptake and cholesterol enrichment of BC cells. These findings were somehow surprising as IFNg has mostly anti-tumoral functions in BC, and increased cholesterol uptake is mainly pro-tumoral. However, a previously described pro-tumoral function of IFNg is the induction of PD-L1 expression. As such, we then tested how increased cholesterol uptake affects PD-L1 surface expression in BC cells. We observed that, surprisingly, exposing BC cells to LDL alone increases PD-L1 surface expression and, according to our hypothesis, IFNg and LDL added simultaneously, further increases PD-L1 at the cell surface – suggesting that both molecules act synergistically. Finally, we show that LDL induces IFNg receptor surface expression and downstream signaling.

Conclusions: Taken together, our data suggest the existence of a positive feedback loop between IFNg signaling and cholesterol uptake through LDLR, which sustains PD-L1 expression in BC cells. This increases our knowledge of how cancer cell metabolism is affected by tumor microenvironment components. It also shows that cholesterol metabolism plays a role in tumor cell interaction with the immune component of the tumor microenvironment. It further supports that controlling systemic LDL levels in patients with BC may be important for the outcome of the disease.

56. Patient-derived organoids as a new ex vivo model to study the role of glycans in gastric pathogenesis

Authors and Affiliations

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Abstract

Glycosylation is a key component associated with stomach inflammation triggered by Helicobacter pylori and the development of gastric cancer (GC). Patient-derived organoids (PDO) are promising ex vivo models for the study of human gastric disorders. Despite their potential, little is known about their potential use to study glycosylation in gastric pathogenesis.

This work aims to validate gastric PDOs as avatars of the glycosylation profile of in vivo tissues. For that, PDOs were established from fresh gastric mucosa (n=10), adjacent tumor mucosa (n=14), and tumor tissues (N=16). Additionally, PDOs derived from the gastric epithelium of C57BL/6 wild-type and Gcnt1-/- mice were also established. The glycophenotype of PDOs and respective in vivo tissues was assessed by IHC analysis using a panel of antibodies and lectins. To validate that tumor PDOs accurately mimic the in vivo histologic features, we establish patient-derived tumor organoid xenografts (PDOX).

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Knowing that gastric epithelium glycosylation plays an important role in H. pylori adhesion – one of the major risk factors associated with GC development – we also evaluate the impact of PDO glycoprofile in H. pylori binding. The interaction between H. pylori and with gastric tissues and PDOs was assessed using two H. pylori isogenic 17875 strains.

Our results showed that PDOs faithfully recapitulate the glycosylation profile of the corresponding in vivo tissue. Importantly, tumor PDOs also replicate the heterogeneity and histological features observed within tumor tissues. We were also able to demonstrate that the glycosylation profile of normal and adjacent PDOs mirrors the human secretor status and influences H. pylori binding mimicking the in vivo conditions.

These findings underscore the crucial role of glycosylation in gastric pathophysiology and highlight PDOs as valuable tools to explore the interplay between glycosylation and gastric pathogenesis.

57. 3D Models of immunosuppressive breast tumor microenvironment to evaluate novel antibody-based therapies

Authors and Affiliations

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Abstract

HER2+ and triple negative breast cancers (TNBC) are characterized by immunosuppressive tumor microenvironments (IS-TMEs), sustained by crosstalk of tumor, myeloid (e.g., tumor-associated macrophages - TAMs) and stromal cells (e.g., fibroblasts). The IS-TME has been proposed as a key barrier to the anti-tumor activity of antibody (Ab)-based immunotherapies. For their preclinical evaluation, it is crucial to employ human-based models recapitulative of the cellular heterogeneity and molecular crosstalk of the IS-TME in BC. Such models must be amenable for molecular interrogation and evaluation of the kinetics of response.

We implemented 3D cell models of the BC IS-TME by co-culturing HER2+ or TNBC cell lines (HCC1954, BT474; HCC1806, MDA-MB-231, respectively) as spheroids, with fibroblasts and monocytes, in alginate capsules. Encapsulated cells built-up IS-TMEs, with extracellular matrix deposition and accumulation of secreted soluble factors. Monocytes differentiated into macrophages with a CSF1R+/DCSIGN+/CD163+/CD206+ phenotype, typical of IS-TAMs. As a proof-of concept of preclinical potential, co-cultures were challenged with trastuzumab (Tmab)-based anti-HER2 Ab-drug conjugates (ADCs), targeting tumor cells, or with anti-CSF1R Ab, targeting TAMs. ADC-induced cytotoxicity was evaluated by cell death (LDH release and caspase activity) and the immunomodulatory effects of α-CSF1R were assessed by flow cytometry. ADCs induced time- and dose-dependent cytotoxicity specific to HER2+ cells, sparing HER2- fibroblasts. α-CSF1R reduced IS-TAMs (83,35±19.42% decrease compared to control Ab), while preserving pro-inflammatory TAMs (CD80+/CD86+).

We developed 3D cell models representative of IS-TMEs of BC, suitable to assess the potential of novel Ab-based therapies. Perspectives include mechanistic insights of resistance to immunotherapies.

58. Inability of mean corpuscular volume change to predict time to treatment failure for treatment with CDK4/6 inhibitors

Authors and Affiliations

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Abstract

Background: Cyclin-dependent kinase 4/6 inhibitors (CDKi) are now the first line treatment for metastatic luminal-like breast cancer (BC). These drugs induce macrocytosis in most patients, the significance of which is not yet clear. In 2020, Choong et al. analyzed the impact of the mean corpuscular volume (MCV) changes caused by CDKis on treatment efficacy. The rationale was that a higher MCV (or faster MCV variation) is due to a more efficient inhibition of the cell cycle by the CDKi. Although the total MCV increase was not predictive of response to treatment, the variation of MCV (Δ MCV) between cycles 2 and 3 was associated with a longer time to treatment failure (TTF). We aimed to validate these findings in an external cohort.

Materials and Methods: Single-center retrospective study. We included patients treated with palbociclib or ribociclib between January 2017 and March 2022 for metastatic BC. Dates of treatment cycles and MCV values were gathered from patient records. An MCV determination with more than 4 days of difference to the starting date of cycles 2 and 3 was an exclusion criterion. Patients were dichotomized according to the Δ MCV from cycles 2 to 3 using the cutoff established by Choong et al (2.9 fL). TTF was defined as the difference between the starting date of the last treatment cycle of CDKi and the start date of the last cycle of CDKi. Survival curves were visualized using Kaplan-Meier plots and hazard ratios were compared using the logrank test.

Results: We identified 64 patients meeting the inclusion criteria. Median age was 60 years. 36 patients with high ΔMCV and 28 with low ΔMCV. Visual inspection of the survival curves as well as the logrank test (p = 0.62) didn't show a statistically different TTF between the two groups.

Discussion and Conclusions: We didn't find a statistically significant difference in TTF between both groups. Our results thus fail to validate the variation in MCV as a predictive biomarker of drug continuation and efficacy. Further studies with larger cohorts are needed to help validate or disprove this potential biomarker.

59. The Role of Transmembrane BAX Inhibitor-1 Motif-containing (TMBIM) Intracellular Ion Channels in Glioma

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Abstract

Considered the most aggressive type of glioma, glioblastoma (GB) is highly invasive and has a low patient survival rate. For these reasons, the development of novel therapies and progression biomarkers is needed. The Transmembrane BAX Inhibitor-1 Motif-containing family (TMBIM) includes 6 highly conserved intracellular ion channels. Several of these proteins can contribute to the control of cellular processes that are central to various hallmarks of cancer. Our work aims at exploring the impact of TMBIM1-6 in glioma progression.

The levels of TMBIM1-6 mRNA in gliomas and their correlation with patient survival was assessed using the TCGA, CGGA and Rembrandt datasets. This analysis revealed a significant upregulation of TMBIM1, 4 and 6 mRNA in gliomas when compared with normal tissues and expression was particularly high in GB. Additionally, a strong correlation between tumour grade and expression levels was observed for TMBIM1, 4 and 6. Importantly, increased levels of TMBIM1, 4 and 6 mRNA were associated with a reduction in the survival of glioma patients. The high level of TMBIM4 expression on the tumour hyperplastic blood vessels and microvascular proliferating regions identified using the Ivy_GAP platform, may suggest the involvement of TMBIM4 on tumour cell invasion along the vessels. To assess the impact of TMBIM4 expression on glioma cell invasion, the U87-MG and U251-MG cells were used as GB models. The knockdown (KD) of TMBIM4 induced a strong inhibition of 2D and 3D cell invasion without affecting collective cell migration, viability, or proliferation. Additionally, the KD of TMBIM4 greatly reduced in vivo tumour growth in a mouse orthotopic model of human glioma.

Overall, our results suggest that TMBIM proteins, particularly TMBIM4, may represent new druggable targets and/or biomarkers for glioma progression.

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60. Recapping Osteosarcoma in vitro: a 3D tool for disease understanding and identification novel druggable targets

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Abstract

Osteosarcoma (OS) the primary bone malignancy most common in children/young adults. OS treatment has virtually remained unchanged for the past four decades, resulting in a stagnant 5-year rate survival of 70% for localized disease and only 20% for metastatic cases. Tumor heterogeneity along with the lack of knowledge about OS biology has hamper the development of novel therapies, exposing the need for the development of predictable 3D in vitro models that can recapitulate the tumor microenvironment (TME), opening new avenues for disease understanding and identification of specific druggable targets, fostering pioneer treatments.

Novel bioengineering solutions, as "tumor-on-chip" platforms, offer a relatively simple TME recreation. Allied to novel disruptive methodologies of prototyping, fabrication, and high-throughput, "on-chip" platforms enable cells/tissue compartmentalization, dynamic flow, ECM inclusion, and facile data analysis.

Here, we propose the development of a fully humanized OS tumor-on-chip (ToC) platform, using a multi-compartment microfluidic chip to co-culture key players of tumor progression and metastasis: OS tumor (composed of tumor, immune and stromal cells), the vasculature (endothelial cells), the lung (epithelial cells) and the ECM, thus recapping, in vitro, the cell-cell/-ECM interactions.

For that, we have established, in vitro, an immune competent OS spheroid using two different OS cell lines, immune cells and mesenchymal stem cells. Spheroids were fully characterized (cellular population, ECM markers, and response to external stimuli (pro-tumoral and anti-tumoral modulation of spheroid behavior). When combined on-chip, OS spheroids was shown to interact with the vascular compartment, namely to intravasate from one compartment to another. Multiomics analysis is currently being employed to unravel potential markers of the OS tumor-vascular crosstalk. Also, the role of immune cells' polarization on OS spheroids growth and invasion capacity is being addressed.

61. HIF1A-associated immunomodulation at the colon cancer microenvironment

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Abstract

Introduction: Colon cancer (CC) is one of the deadliest cancers, and depending on the location, tumors have very different presentations and prognosis.

In CC, the role of macrophages is controversial, with some authors claiming that high infiltration is associated with better prognosis, while others report that their presence is associated with increased tumor progression.

We previously shown that hypoxia modulate the crosstalk between cancer cells and macrophages, and reported that HIF1A levels impact on patients' survival.

Aim: To explore whether HIF1A expression is associated with differences in patients' survival depending of the tumor location on the right or left colon, and on macrophages infiltration levels.

Methods: Data from CC patients available at the COAD database from The Cancer Genome Atlas (TCGA) was used. As an indication of macrophages infiltration CD68 expression was used, and the tumors divided between left or right colon. Among the groups, survival differences between patients with high or low HIF1A expression was assessed using Kaplan-Meier curves. CIBERSORT analysis was performed to evaluate differences in the immune populations presents at the tumor microenvironment, and the expression levels of immune checkpoints molecules were analyzed.

Results: Within CD68High patients, HIF1AHigh tumors present better prognosis than the HIF1ALow, only in right colon. When comparing groups with differences in survival, we found that there were differences not only in the immune profile of the tumor microenvironment, but also in the expression profile of different immune checkpoints, some of which are associated with a better anti-tumor immune response.

Conclusions: HIF1A may have an important role in the immunomodulation of the CC microenvironment. Whether the HIF1A is related with the expression of immune checkpoints associated with an anti-tumor immune response should be studied, as well as the context of its activation, in hypoxic or non-hypoxic conditions.

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62. The role of ERα, AR and tumor microenvironment for the aromatase inhibitors (AIs) used in breast cancer treatment

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Abstract

Introduction: Around 70-85% of all breast cancer (BC) cases are estrogen receptor-positive (ER+). The third-generation of aromatase inhibitors (AIs), anastrozole (Ana), letrozole (Let) and exemestane (Exe) are the first-line treatment option for these tumors, in postmenopausal women or in pre-menopausal women, after ovarian function suppression. Despite their success, resistance may occur, which highlights the importance of understanding the mechanisms induced by these AIs on BC cells in order to find new targets/strategies.

Methods: Using MCF-7aro cells it was studied the expression and activation of $ER\alpha$ and AR (MTT, Western-blot and qPCR). Using VM7Luc4E2 cells and CHO-K1 AR-transfected it was assessed the agonistic/antagonistic activity on ER and AR, respectively (ER and AR Transactivation Assays). Cells treated with AIs were stimulated with testosterone or estradiol for tumor microenvironment analysis.

Results: Results showed that, even though Ana and Let present antagonistic behavior on $ER\alpha$, they act as pure AIs on BC cells. Curiously, besides acting as an AI, Exe also has the ability to modulate both $ER\alpha$ and AR, exerting on AR an agonistic pro-survival effect, while on $ER\alpha$ it acts as an antagonist or agonist depending on tumor microenvironment, which influences its pro-death/pro-survival effect.

Conclusion: Our work provides new insights on the mechanisms induced by the AIs on BC cells, highlighting new targets to improve treatment. It discourages the sequential use of Exe as second-line therapy in postmenopausal BC, emphasizing their beneficial use as first-line. Moreover, it may also support the ongoing clinical trials with AIs and AR antagonists, namely with Exe, and highlights the non-application of selective estrogen receptor down-regulators (SERDS) with AIs.

63. Inhibition of CCR2 as a potential therapeutic approach to treat medulloblastoma

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Abstract

Medulloblastoma (MB) is the most common malignant pediatric brain tumor. MB can be classified into four molecular subgroups, namely WNT, SHH, Group3 and Group4. MB frequently forms metastases within the central nervous system which aggravate patient prognosis and outcome. Group3 and Group4 MB have the higher incidence of metastases. Current therapies effectively treat the primary tumor, however metastases remain incurable. Recent studies suggested an association between the CCL2/CCR2 axis and MB progression. Here we hypothesized that CCR2 is a prognostic marker of MB and that inhibition of the CCL2/CCR2 axis can treat metastatic MB.

To test our hypothesis, we assessed the clinical relevance of CCR2 gene expression in MB patients using the R2: Genomics Analysis and Visualization Platform. Additionally, we overexpressed the CCR2 gene in the ONS76 MB cell line (ONS76 OE-CCR2) and evaluated its effect in cell proliferation and migration. Moreover, using these cells we established an orthotopic mouse model and assessed how CCR2 gene expression affects tumor invasion and dissemination, as well as animal survival. Finally, we performed an in-silico drug screen to identify inhibitors of CCR2 and studied their efficacy in treating MB progression, both in vitro and in vivo.

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Higher expression of CCR2 associates with dissemination and reduced survival in pediatric Group3 MB patients. CCL2 stimulation increased ONS76 OE-CCR2 cell proliferation and migration. Overexpression of CCR2 in MB cells increases tumor invasion and decreases survival of mice. In our in-silico drug screen we identified Cenicriviroc, Marizomib, and Romidepsin as potential CCR2 inhibitors. Cenicriviroc decreased CCR2 expression, reduced cell proliferation and migration, and inhibited CCR2 signaling. Mice treated with Cenicriviroc presented a significant decrease in tumor growth.

In summary, we believe that CCR2 is a potential marker of MB prognosis and inhibition of CCR2 holds promise for treating metastatic MB.

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64. Sensitizing pancreatic cancer to immunomodulatory therapies through impaired extracellular vesicles secretion via Rab27a loss

Authors and Affiliations

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Abstract

Inoperable pancreatic ductal adenocarcinoma (PDAC) patients face an average survival of 11 months, underscoring the need for novel therapeutic approaches. While immunotherapy holds transformative promise in cancer care, its effectiveness in PDAC is limited. Here, we investigated the potential of extracellular vesicles (EVs) secreted by cancer cells (cEVs) in modulating the immune response against PDAC. Our study focused on disrupting cEV-mediated communication as a strategy to sensitize PDAC tumors to immunomodulatory therapies.

To accomplish this, we developed a genetically engineered mouse model that impairs cEVs secretion via Rab27a knockout (KO) in a PDAC context. Interestingly, KO mice displayed earlier disease onset and reduced overall survival, particularly in an immunocompetent background, suggesting immune system involvement. Further analysis revealed dysregulated immune response and inflammation, driven by MRP8+ myeloid and Th17 T cells. Mechanistically, we found that MRP8 prompts cancer

associated fibroblasts to secrete pro-inflammatory cytokines, driving CD4+ T cells toward a protumorigenic Th17 phenotype. We also noted that loss of TSP1+ cEVs is critical to these changes by mediating CD11b+MRP8+ myeloid cells recruitment. Anti-inflammatory therapy and CD4+ T cell depletion mitigated aggressive KO tumor traits, with no impact on Rab27a wild type tumors. In human PDAC, Rab27a downregulation was associated with disease progression, and 24% of patients lacked Rab27a expression. Notably, patients with low Rab27a and high MRP8+ cell infiltration faced poorer prognosis. Molecularly, we identified SMC3 methylation, a cohesion complex component, disrupted chromatin folding, impairing Rab27a cis-regulation, thus inhibiting gene expression in PDAC.

Our work unveils a tumor suppressive role of cEVs in PDAC and deciphers the underlying mechanism. Importantly, we identified a subset of PDAC patients that could benefit from an immunomodulatory therapeutic approach.

65. The immune system shapes the tumor genetic landscape skewing against putative immunogenic mutations

Authors and Affiliations

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Abstract

Introduction: Cancer immunosurveillance is a dynamic process responsible for the elimination of neoplastic cells. Its efficacy depends on the ability of the immune system to detect and eliminate cells expressing immunogenic neoantigens, which arise from somatic mutations. In fact, tumors with replication error repair deficiency (RER+) have been correlated with improved responsiveness to immunotherapies, possibly explained by the increased neoantigen load resultant from a higher mutation burden. However, the identity of the immunogenic neoantigens remains largely unknown. We took advantage of a model of immunocompetent (IC) and immunodeficient (ID) RER+ mice, where lack of proficient adaptive immunity results in increased tumor incidence, to address this question.

Materials and Methods: Intestinal neoplastic lesions and skin tissue from both IC VCMsh2LoxP/LoxP and ID VCMsh2LoxP/LoxPRag2-/-IL2rg-/- mice were collected. DNA was extracted and used to identify tumor-specific somatic mutations through whole exome sequencing, excluding germline variants that were shared by skin-derived DNA. Validation of somatic mutations was done through Sanger Sequencing in a larger group of mouse intestinal tumors. To observe if our results were translatable to the clinics, a mutational screening was performed in a cohort of 80 human colorectal cancer (CRC) samples.

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Results: Tumors from ID mice showed a significantly higher mutation burden when compared with tumors from IC mice. Also, Asxl1, Ctnnb1 and Rgs12 genes were found to be significantly more mutated in mouse tumors growing in an immunodeficient context. Moreover, these genes were also found to be mutated in human CRC.

Conclusion: The differences in mutation burden and gene-specific mutation frequency in tumors from ID and IC mice result from a selective effect of adaptive immunity on neoantigens. Understanding this process has the potential to uncover new or improve current immunotherapeutic approaches.

66. Metabolic modulation of tumours via peptide nanotechnology

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Abstract

Introduction: Dysregulated amino acid metabolism is known to be implicated in supporting cancer development. While cancer cells use essential amino acids to sustain their proliferative rate, their consequent depletion in the tumour microenvironment (TME) has also been shown to impair anti-tumour immunity. Thus, metabolic modulation of tumours for enhanced immunotherapy is receiving increasing attention. We propose a peptide nanotechnology approach to release key amino acids in-situ, when exposed to proteolytic enzymes present in the TME, and boost the efficacy of cancer immunotherapies.

Materials and Methods: To test our hypothesis, we have rationally designed peptide amphiphiles (PAs) with an aliphatic chain at the N-terminus and arginine at C-terminus (C16Ala6-Arg3 and C16Ala6-His2-Arg). The PAs were synthesized via solid phase peptide synthesis, and their identity and purity verified by LC-MS and reverse phase-HPLC, respectively. Surface charge, critical aggregation concentration, and structural characterization were carried out via Zeta potential, Thioflavin-T assay, CD, and TEM, respectively.

Results: Spectroscopic and microscopic studies have shown the intermolecular arrangement of PAs into fiber-like nanostructures with a positive surface charge due to the terminal arginine. Furthermore, the sequential degradation of arginine was quantified by LC-MS. The cytotoxicity of PAs against selected cancer cell lines and macrophages is under investigation.

Conclusions: Our preliminary experiments show the possibility to feed key amino acids in the TME at controlled rate and future studies will investigate the ability of released amino acids in potentiating the anti-tumour response of immune cells.

67. Tumor educated platelets signature allow to identify and predict the survival of multiple myeloma patients

Authors and Affiliations

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Abstract

Multiple myeloma (MM) is a clonal plasma cell disorder genetically complex, with a high biological and clinical heterogeneity. MM diagnosis and management is highly dependent on bone marrow aspirate, a relative invasive and painful procedure, that does not capture the spatial and clonal heterogeneity of this disease. The role of tumor-educated platelets (TEPs) as a source of peripheral specific biomarkers has been showing promising results for cancer management. However, its role in hematological diseases is still poorly understood.

The aim of our study was to identify in MM patients a TEP gene expression signature and correlate it with patients' survival data.

To achieve our goal two different GEO databases were used. The GSE183635 that includes RNA-sequencing data of platelets from 22 MM patients and 183 asymptomatic healthy donors (aHD); and the GSE24080 with gene expression microarray data of CD138+ cells and survival data of 559 MM patients. All the analyses were performed using R and R Studio (version 2023.06.1) and different R packages (GEOquery, limmaVoom, edgeR, ggplot2, etc).

A total of 223 differentially expressed genes were identified between platelets from MM patients and aHD. Nineteen genes presented higher statistical significance with p-value <0.000001 and a fold change >2 in MM patients. Among these, 15 were over expressed (HBD, CA1, GZMH, IFI27, LY6E, ITGB7, ITGAL, NKG7, RAD23A, NADSYN1, DUSP2, PAXX, GZMB, TCIRG1, and OSBPL5) and 4 were under expressed (WDR11-DT, ZNF385D, GRHL1, and HSD17B3).

The prognostic value of these 223 genes, generated as a MM TEP signature, was then explored. We found that 97 were significantly correlated with MM survival (p<0.05). Moreover, 11 genes (EFHD2, CMTM6, ARPC5L, TNNC2, GSTP1, RASAL3, GAK, ANGPT1, DNAJC7, PLAAT4 and TLR5) presented a higher impact on MM prognosis with p<0.001 and hazard ratios higher than 1.5 (1.636±0.119).

Our results showed that TEP mRNAs were differentially expressed in MM patients compared with aHD, and that this new MM TEP signature might be used as a screening test and prognostic biomarker in MM patients.

68. Piecing together the CDH1 regulatory puzzle: A path to early gastric cancer onset

Authors and Affiliations

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Abstract

Background: Hereditary diffuse gastric cancer (HDGC) is caused by CDH1 or CTNNA1 germline inactivation, which explains <40% of cases. Missing heritability prevents proper management and disease prevention in HDGC-suspected families. We hypothesized that defects in CDH1-regulatory elements contribute to HDGC missing heritability.

Methods: We called single-nucleotide/copy-number variants (SNV/CNV) from 19 HDGC probands whole-genome sequencing data, performed gene-ontology analysis, 4C-seq and ATAC-seq in normal stomach epithelia and CRISPR-Cas9, RT-PCR and flow cytometry in cell lines.

Results: Besides a MLH1 2,7kb deletion, no relevant coding variants were found in gastrointestinal cancer-associated genes. From stomach ATAC and 4C-seq data, we extracted 46.249 accessible chromatin regions and 370 promoter CDH1 interactor-regions overlapping 1.882 rare CNVs. CNVs overlapping CDH1 promoter interactions revealed a 39bp-intergenic deletion downstream of CDH1, and a 20kb CDH3 deletion. CRISPR-Cas9 mimicking each deletion (homozygous) triggered 30% and 50% CDH1 mRNA downregulation, respectively for the 39bp-intergenic and CDH3 CNVs. These sequences acted as enhancers in E-cadherin expressing tissues during development of mouse embryos. The pattern of deleted accessible chromatin regions per patient revealed a HDGC-group bearing downregulated immune-associated pathways.

Conclusion: We identified two novel hypomorphic CNVs in CDH1-regulatory regions contributing for CDH1 downregulation, and a MLH1 deletion in a family without classical Lynch Syndrome, that may explain the missing heritability in three HDGC-suspected families. A gastric-specific regulatory elements within the MLH1 CNV support predisposition to gastric cancer in this family. Germline CNVs in stomach-specific regulatory regions may predispose to a immune-suppressive phenotype favourable for HDGC developement.

69. Nano-immunotherapy for melanoma – a window of translation oncology

Authors and Affiliations

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Abstract

Cancer research in dogs offers an opportunity for translational oncology, as this species shares deadly characteristics with human counterpart. Melanoma has inherent immunogenicity and express specific antigens (e.g., Gp100, MelanA) explored as immunotherapeutic targets. Here we report a dendritic cell-target nano-immunotherapy previously validated in human melanoma.

Following an immuno-bioinformatic analysis, we identified five peptide sequences (antigens) of Gp100 and MelanA suitable to trigger CD8+ and CD4+ T-cell activation. These peptide sequences were co-entrapped together with immune potentiators (Poly(I:C) and CpG – TLR agonists) in a polymeric-based nanoplatform (NP). After physical and chemical characterization, the candidate was Gp100-NP. A preliminary in vivo study using the B16F10-melanoma model was performed to assess T-cell activation with canine Gp100-NP. Animals were immunized two times 7 days apart. On day 22 the spleens were then recovered and processed accordingly for immune-cell profiling.

We showed that Gp100-NP activated the CD8+ and CD4+ T cells, and triggered antigen-specific cytokine (TNF-α, IFN-g and IL-2) secretion, when compared with non-immunized and antigens in solution. Further steps are currently taking place to establish a primary culture of canine immune cells to assess specific immunogenicity.

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70. Intestinal stem cell marker MEX3A acts as a PPARγ direct regulator with functional impact in colorectal carcinogenesis

Authors and Affiliations

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Abstract

Introduction/Objectives: RNA-binding proteins (RBPs), as part of the post-transcriptional regulatory machinery, play a key role in diverse cellular processes. Recently, we described the role of MEX3A RBP in maintaining LGR5+ intestinal stem cells identity and epithelial renewal by repression of the PPAR pathway. This work aimed to characterize MEX3A functional impact in colorectal cancer (CRC) and uncover MEX3A targets.

Methods: We characterized the molecular expression profiles of CRC mouse models and a cohort of CRC cases (n=172). Mouse CRC tissues were used for the establishment of tumoroids (MDTs), and MEX3A CRISPR/Cas9-mediated knockout was performed in patient-derived tumoroids (PDTs) to further understand its biological relevance. Simultaneously, we implemented the high-throughput technique HyperTRIBE to uncover MEX3A RNA targets.

Results: Intestinal adenomas from Apc+/fl mice have increased expression of Mex3a and low PPAR expression. Apc+/fl;Mex3a+/- animals presented a significant reduction in tumour burden. Apc+/fl;Kras+/G12D;Mex3a+/- compound mice presented reduced tumour area, and MDTs exhibited reduced growth ability and enhanced differentiation potential mediated by PPAR signalling. MEX3A overexpression was observed in 85% of human CRC cases, while 72% presented PPAR downregulation, with a statistically significant inverse correlation (P=0.039). Accordingly, MEX3A-depleted PDTs showed decreased Lgr5 expression, accompanied by increased PPAR expression. HyperTRIBE results revealed a direct interaction between MEX3A and PPARG transcripts.

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Conclusion: Our results suggest that MEX3A overexpression has an important role in CRC carcinogenesis by repressing the PPAR differentiation pathway.

71. Dissecting the role of PGRMC1 as a potential CSC biomarker associated with breast cancer metastasis

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Abstract

Breast cancer (BC) is a major cause of cancer death, mainly due to distant metastases. Although cancer stem cells (CSCs) represent a small subpopulation within the tumour, they are described as being involved in metastasis formation. Therefore, the search for new CSC biomarkers and improvements in CSC-targeted therapies are crucial to tackle metastatic progression. In this work, our major goal was to specifically identify membrane CSC biomarkers and dissect their potential role on breast cancer metastasis.

For that, the MDA-MB-231 metastatic BC cell model and its organotropic variants for bone, brain and lung were used. Through membrane proteomic analysis, PGRMC1 was identified as a biomarker of CSCs potentially associated with metastatic organotropism. After PGRMC1 chemical inhibition with AG-205, a significant decrease in mammospheres formation was observed for the brain organotropic variant, confirming an impact on the stem properties of BC cells with organotropism in vitro. Importantly, using a cohort of primary breast tumours, high PGRMC1 expression was associated with clinicopathological features, as well as with the expression of biomarkers associated with poor prognosis in BC. Moreover, a significant association was observed between high PGRMC1 expression and a worse 5-year disease free survival.

In summary, PGRMC1 not only showed to impact on the stem properties of BC cells with organotropism in vitro, but also associated with a worse prognosis for BC patients. Therefore, further work should be performed to consolidate the relevance of PGRMC1 not only as a biomarker for CSCs, but also as a prognostic factor and therapeutic target.

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72. The effect of gut microbiota manipulation in two colorectal cancer mouse models

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Abstract

Colorectal cancer is the 3rd most incident and the 2nd most lethal cancer worldwide. Radiotherapy and chemotherapy are the leading treatment options, and immunotherapy has emerged as a new approach. Despite the beneficial effects observed, the response to immunotherapy is low. Recent evidence showed that the gut microbiota can both influence cancer development and predict response to immunotherapeutic drugs in cancer patients. Therefore, our aim was to use colorectal cancer as a model to explore the triangular relationship between the microbiome, the immune response, and cancer.

We modulated the gut microbiome of two genetically modified mouse models that recapitulate human colorectal cancer, an Apc-driven model mimicking the chromosomal instability phenotype and a Msh2-driven model mimicking the microsatellite instability phenotype. Mice were treated with an antibiotic cocktail to deplete the gut microbiota, inoculated with Bifidobacterium species, and tumorigenesis was then induced. Faeces were collected for gut microbiome analysis by next-generation sequencing and qPCR. Intestinal tumour tissues were collected for histopathological evaluation and for immune profile characterization at the time of euthanasia.Treatment with the antibiotics cocktail resulted in a significant decrease in total gut bacterial load. A significant increase in Bifidobacterium species was also observed after bacterial inoculation. Preliminary data shows that Bifidobacterium-inoculated Apc-mice had lower number of tumours compared to control- and gut microbiota-depleted animals. In Msh2-mice, Bifidobacterium-inoculated animals showed decreased tumour incidence. Further experiments are ongoing to characterize the immune profile of the tumours.

73. Biochemical and biomechanical characterization of the extracellular matrix of colon cancers reveals tumor and side-specific features

Authors and Affiliations

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Abstract

Introduction: The extracellular matrix (ECM) is a dynamic entity that is likely to play an important role in cancer progression. In colon cancer, high stromal content is associated to poor patient prognosis. However, it is still unclear what is the specific contribution of the ECM for this phenomenon. Therefore, we aimed to dissect the differences in ECM properties between non-malignant and malignant colon tissues while considering the sidedness of the tumors.

Materials and Methods: Paired normal and tumor tissues (n=12) from right and left colon were decellularized and analyzed by label-free mass spectrometry (LC-MS/MS) and rheology. ECM morphology considering fiber density and orientation was investigated by polarized light and multiphoton microscopy, respectively. Differential features between normal and tumor tissues and between tumor location were explored by bioinformatic analyses.

Results: Tumor ECM was stiffer than normal matrix going in line with increased fiber density and upregulation of proteins involved in fiber formation. Likewise, single-sample pathway analysis revealed an enrichment in TGF-beta signaling and elastic fiber formation pathways in tumor ECM which infer fibroblast-associated activity. Interestingly, these differences showed to be mainly driven by left-sided colon samples. Also, left-sided tumors were stiffer then right-sided tumors accompanied by higher fiber density, showing that while right tumors have an ECM profile more similar to normal tissue, left tumors are a distinct entity. Finally, tumor ECM fibers were less tortuous and more oriented than normal fibers in both locations, and proteomics differences were revealed.

Conclusions: This study highlights not only tumor but also side-specific ECM features, a novelty in the field that might contribute for the distinct molecular profile and clinical management in right- and left-sided tumors. Therefore, considering these players in future studies will bring new insights into colon cancer biology and potential novel biomarkers.

74. Matrix metalloproteinase activity modulation in glioblastoma through a novel nanotherapeutic strategy

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Abstract

Current glioblastoma (GB) treatments are largely ineffective, with patients´ median survival of only 15-20 months. The blood-brain barrier (BBB) limits molecule access and overexpression of matrix metalloproteinases (MMPs) contributes to GB's invasiveness. Temozolomide, the standard treatment, faces challenges like poor solubility and lack of specificity for GB. Therefore, alternative compounds and delivery strategies are urgently needed.

In this work, we employed a targeted Nanostructured Lipid Carrier (NLC) to deliver Batimastat, a potent MMP inhibitor, for treating GB. The NLCs were functionalized with epithelial growth factor (EGF) and an MMP-14-cleavable pegylated transferrin-mimicking construct (MMP14cp-PEG-TfP), for both BBB crossing and GB microenvironment entrapment.

Blank NLCs (bNLC) were synthesized by an ultrasonicator-assisted hot homogenization technique, with Batimastat added during the melting process. Functionalizations (fNLC) were achieved through carbodiimide chemistry. All NLCs were characterized for size, polydispersity index (PDI) and ζ potential by dynamic/electrophoretic light scattering, morphology by transmission electron microscopy, encapsulation efficiency (EE%) by high-pressure liquid chromatography (HPLC), and functionalization efficiency by micro-BCA.

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fNLCs presented a size of 193.4 ± 48.8 nm, PDI of 0.200 ± 0.053 and ζ potential of -21.2 ± 1.5 mV, with 85 ± 15 % functionalization efficiency. Batimastat EE% in bNLCs was 30.5 ± 0.6 % and the EE% in fNLCs is being analyzed.

Our data demonstrates that NLCs encapsulate Batimastat at concentrations up to 3000 nM, a biologically effective level. Moreover, we can enhance NLCs with targeting components to potentially improve GB treatment efficacy. Future research will assess the impact of this nanoformulation on GB cell lines.

75. Data mining of genome-wide CRISPR-Cas9 screens and functional testing to assess ancestry influence in triple negative breast cancer

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Abstract

Introduction: Women of Sub-Saharan (SSA) descent have a disproportionate burden of triple-negative breast cancer (TNBC), for which there is no targeted drug intervention. High-throughput assays, as genome-wide CRISPR–Cas9 screens in extended cancer cell line panels, are valuable for identifying cancer gene essentialities and prioritizing candidate therapeutic targets. Data from these large screens are publicly available for data mining.

Materials and Methods: We selected SSA and European (EUR) TNBC cell lines for the integrated gene effect scores datasets from Broad and Sanger institutions. A differential essentiality analysis was performed using limma and ssGSEA algorithms, and pairwise analyses on mRNA, protein and mutation levels were conducted. We are carrying out functional assays on the candidate hits from the bioinformatic analyses.

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Results: TN SSA cell lines showed a stronger dependency on the BLM gene, a pivotal member of the RecQ family of DNA helicases. Also, at the level of molecular pathways, DNA repair and replication were the top findings of essentiality for SSA. Concordantly, BLM expression was significantly correlated with structural alteration burden in cell lines and patients. There is a tendency for BLM mRNA and protein levels being lower in SSA than EUR cell lines and patients, but values were not statistically significant, possibly indicating fine tuning regulation. We are finalising functional analysis on cellular location and phosphorylation status of BLM and interacting proteins on TNBC cell lines of SSA and EUR ancestries. And will contextualise these results in terms of genomic instability (micronuclei quantity) and innate immune impact.

Conclusions: We identified BLM essentiality in TNBC of African origin and will provide functional evidence to elucidate its biological underpinnings. These results will be relevant to explore novel target mechanisms for TNBC treatment.

76. Nano-immunotherapy for tumor immune microenvironment regulation against solid tumors

Authors and Affiliations

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Abstract

Despite the remarkable efficiency of immune checkpoint modulators against metastatic cancer, there is a low percentage of responders and clinical trials severe side effects and disease relapse. Recent evidence shows that non-tumor cells within the tumor microenvironment (TME), including tumor vasculature and stromal cells, dictate the therapeutic efficacy in aggressive tumors, such as pancreatic cancer and triple negative breast cancer. We are developing precision nano-medicines aim to re-educate and harness patient T-cell response against tumors, yielding an immunological memory able to control tumor relapse.

Polymeric nanoplatform (NP) was synthesized to co-entrap tumor-associated antigens, toll-like receptor ligands and regulators of PD-1/PD-L1 pathway and cytokines (TGF-β) identified as major drivers of melanoma, breast cancer and pancreatic cancer progression. NP internalization and dendritic cell (DC) activation profiles were evaluated by flow cytometry. The immunotherapeutic potential of the three NP was assessed in mouse models of melanoma (B16F10), breast cancer (4T1, E0771) and pancreatic cancer (KPC).

NP induced DC activation and maturation, significantly increasing the expression of CD80 and CD86 surface markers. Our first NP candidates remodeled the TME of melanoma, breast cancer and pancreatic cancer-bearing mice, significantly delaying tumor development and increasing disease-free survival rates. Our NP led to the successful eradication of malignant cells by restoring T-cell activation and thereby enabling the induction of an effector and memory anti-tumor immune response. This innovative approach discloses the ability of our multifunctional NP to restore tumor microenvironment immunity by regulating stromal vasculature and immune cell function.

77. CDK4/6 inhibitors and erythropoiesis – computational insights into hematological effects

Authors and Affiliations

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Abstract

Background: CDK 4/6 inhibitors (CDKi) are the first-line treatment for metastatic luminal-like breast cancer (BC). They induce macrocytosis without anemia in most patients. The mechanism for the red blood cell (RBC) changes is unknown. In vitro studies show that CDK6-knockout RBCs have increased membrane fragility. The clinical impact of CDKis on human RBC lifespan is not known. Using the mean corpuscular volume (MCV) measurements at several time points, we can study the evolution of MCV, mean corpuscular hemoglobin concentration (MCHC) and RBC count over time. From this, one can estimate RBC lifespan under CDKi.

Materials and Methods: Retrospective study of BC patients treated with CDKi between 2017 and 2022 for metastatic disease (any line). Patients were identified from pharmacy records. Laboratory measurements were extracted from the patient's electronic medical records. Based on data published on RBC population dynamics, we've coded a biologically inspired hierarchical Bayesian model in the Stan programming language. At each point in time, the MCV was modeled as a weighted average of the MCV of the pre-treatment RBCs and the post-treatment RBCs. We fit the model to the data and extracted 95% Bayesian credible intervals (CdIs) for the model parameters, including the time delay.

Results: A total of 122 patients were identified and 1959 lab measurements were analyzed. After the pre-treatment RBCs were replaced, the mean MCV increased by 12.6fL (95% CdI 13-14), the MCHC increased slightly by 0.69g/dL (95% CdI 0.42-0.96) and the RBC count decreased by 0.77×109/L (95% CdI 0.42-0.96). The net result was a 0.64g/dL (95% CdI 0.48-0.80) rise in hemoglobin. The mean total RBC lifetime was 118 days (95% CdI 114-122) days, similar to the value in healthy persons. The model performed well in diagnostic checks.

Conclusions: The model suggests that CDKis don't decrease the RBC lifespan in pre-treatment erythrocytes. Unfortunately, this method can't determine the lifespan of post-treatment RBCs. The increase in MCV is counterbalanced by a decrease in RBC numbers, in which the net result is a slight hemoglobin increase. These findings may help guide experiments to better understand these hematological effects.

78. Screening the triple negative breast cancer cell glycome to target immunosuppression mediated by tumour-associated macrophages

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Abstract

Recent, new generation immunotherapies show good response in some triple negative breast cancer (TNBC) patients. Still, patients with immunosuppressive tumours often remain refractory.

Increasing evidence implicate aberrant glycosylation on TNBC cells in immunosuppression. Specific glycans bind to lectins displayed by tumour-associated macrophages (TAM), driving an immunosuppressive phenotype and immune evasion. We aim to expand the knowledge on the glycan-lectin signatures contributing to immunosuppressive TNBC. We employed a human-based 3D model recapitulative of the TNBC microenvironment, performed systematic glycome screenings and assessed TAM lectin recognition specificities.

TNBC cell lines were aggregated into spheroids and microencapsulated with human primary monocytes in alginate. Surface TAM proteins were assessed by flow cytometry and secreted cytokines by antibody arrays. Glycan epitopes presented on BC cell proteins were profiled by reverse-phase protein and lectin microarrays.

Our results showed increased TAM representation and higher secretion of CCL2, M-CSF and lactate in TNBC compared to luminal BC models. In TNBC models, TAM presented high levels of immunosuppressive surface proteins, including CD163 and the lectins CD206, SIGLEC-9, DC-SIGN and MGL. Together, the data suggest a strong immunosuppressive microenvironment in TNBC models. Glycan screening revealed differential glycan profiles from TNBC spheroids compared to monolayers, with loss of Core 1 and N-acetyllactosamines antigens in 3D and cell line-specific profiles. Noteworthy, known binding partners of TAM: high mannose, fucosylated and sialylated epitopes, were identified. Our approach pinpointed critical glycan cues known to TAM polarization, being currently explored to identify novel biomarkers for TNBC.

79. The integrated stress response releases the oncoprotein in TP53

Authors and Affiliations

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Abstract

Introduction: The TP53 gene is surrounded by a great duality: it is a critical tumour suppressor gene, however, its protective nature is frequently lost in tumours, where it becomes a powerful oncogene. Numerous studies attribute the oncogenic profile of TP53 to the missense mutations that commonly occur in cancer. We propose that this duality is intrinsic to TP53 instead of a consequence of mere somatic mutations, which could not have been evolutionarily selected for. This gene encodes for a set of protein isoforms with distinct and complementary functions, from which the full-length p53 (FLp53) is the best characterized. FLp53 is a transcription factor that mediates stress responses by promoting cell cycle arrest, DNA repair or apoptosis. In stark contrast to FLp53, the shorter isoform Δ160p53 promotes cell survival, proliferation, and invasion, and it is commonly overexpressed in tumours. Here we identify a disruption in the normal balance of p53 isoforms upon induction of the Integrated Stress Response (ISR), with the translation of Δ160p53 being favoured.

Materials and Methods: Different cell lines were used to verify the expression of endogenous p53 isoforms during ISR, and the internal translation of Δ160p53 was tested with bicistronic constructs. The interaction of Δ160p53 with FLp53 was assessed by co-immunoprecipitation followed by western blot, and its effect in the expression of target genes was measured by RT-qPCR. Cells were treated with thapsigargin and tunicamycin to induce ISR when required.

Results: The induction of ISR in a group of cell lines led to increased levels of endogenous Δ160p53 protein, as well as increased luminescence signal in the bicistronic system. The FLp53-Δ160 interaction was confirmed, and the role of Δ160p53 in the selective regulation of p53 target genes was uncovered.

Conclusions: These data uncover a mode of activation of the oncogenic Δ160p53 and how this isoform can work together with FLp53. In the future, we aim to explore the clinical potential of these discoveries.

80. Tumor-targeting strategies for the development of selective therapies: p28 and drug penetration using in vitro 3D cancer spheroids

Authors and Affiliations

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Abstract

Introduction: Conventional systemic cancer therapies induce severe side-effects, which limit their efficacy and safety. In the last decade, extracellular vesicles (EVs) emerged as promising nano-drug delivery systems (NDDS). These display amenability for loading with multiple anticancer therapeutics and engineering aiming to improve their accumulation at the target tissues. The anti-cancer cell penetrating peptide p28 has recently been demonstrated to facilitate the uptake of nanoparticles in lung cancer and deliver efficiently anti-cancer drugs [1].

Materials and Methods: In order to develop an EV-based NDDS for targeted cancer therapy, mesenchymal stromal cell (MSC) derived-EVs were isolated through a GMP-compatible scalable protocol comprising tangential flow filtration (TFF) and size exclusion chromatography (SEC). EVs were decorated with the azurin-derived p28, a peptide displaying preferential penetration into cancer cells. Moreover, the ability of p28 to diffuse through breast cancer spheroids has been tested. MCF-7 spheroids were assembled using agarose molds casted on the 3D Petri Dish® micromolds (MicroTissues Inc., USA).

Results: Incubation of triple-negative breast cancer cells (MDA-MB-231) with EVs-p28 led to an increased cell uptake of EVs by about 1.6-fold in comparison to native EVs. Regarding the p28 penetration, spheroids were generated and fluorescently labeled p28 (25 µM of FAM-labeled peptide over a total of 100 µM) was added at Day 3 and the degree of penetrations was evaluated at Days 4 and 7 using confocal imaging. On Day 7, after 96h of exposition to p28, the peptide is present in the central areas of the spheroid.

Conclusion: Overall, this novel NDDS could be valuable for promoting increased tumor retention of anticancer agents that have associated off-target effects, rendering them more effective. We will now evaluate how the functionalization of EVs with p28 may impact their penetration through the spheroids and influence cells' drug resistance profile.

[1] Garizo AR, Castro F, et al. (2021) J Control Release (337):329-342. doi: 10.1016/j.jconrel.2021.07.035

81. Polyphenol metabolites modulate cell features critical for renal cancer initiation and progression

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Abstract

Plant secondary metabolites, such as polyphenols, have been studied for their benefits and potential therapeutical uses in health, including anti-tumoral activity. Polyphenols are highly present in human diet and have been widely reported for their protective effects against a range of pathologies, including renal cancer. The in vitro methodologies commonly used to explore the effects of dietary polyphenols on kidney cells do not consider the extensive in vivo metabolism of these compounds, disregarding both molecule biotransformation and physiological concentrations. We here investigated the role of polyphenol metabolites (PMs) on phenotypes relevant for cancer development using normal-like (HK-2 and Vero-E6) and tumor (786-O) kidney cell models. Cells were exposed to four PMs belonging to the phenolic acids class (phenylacetic, protocatechuic, homovanillic and dihydrocaffeic acids), at physiological concentrations for 24h. We evaluated the effect of this exposure on cell viability using a crystal violet colorimetric assay. Additionally, we assessed the migratory ability of tumor cells at different levels, including collective migration using the wound healing assay, individual cell migration and invasion using transwell-based approaches, and random cell migration using time-lapse microscopy. Moreover, we addressed the potential protective effect of these PMs on DNA damage upon induction with cisplatin in non-tumor kidney cells. Our results demonstrate that cell viability was not altered upon treatment with PMs in all cell lines. Dihydrocaffeic acid was shown to disrupt cell migration dynamics of tumor cells, particularly affecting cell velocity. Preliminary results are also suggestive that phenylacetic and homovanillic acids may play a protective role against cisplatin-induced DNA damage on non-tumor kidney cells. These findings suggest a potential effect of these PMs in counteracting tumor cell features of great impact for cancer initiation and progression, in a physiological relevant context.

82. immune checkpoints PD-1 and LAG-3 in feline mammary carcinoma metastasis: tumor microenvironment insights and therapeutic implications

Authors and Affiliations

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Abstract

Introduction: Recent research strongly indicates that evaluation of programmed cell death protein 1 (PD-1) and lymphocyte-activation gene 3 (LAG-3) expression holds great promise as a therapeutic strategy for combatting breast cancer. However, scientific data about PD-1 and LAG-3 expression in distant metastatic sites are scarce, despite the tumor immune microenvironment can be heavily influenced by organ-specific parenchymal cells. Feline mammary carcinoma offers a valuable platform for cancer research, also because the postmortem collection of metastatic tissues is more socially accepted.

Materials and Methods: Thus, in this study, we evaluated the expression of PD-1 and LAG-3 by immunofluorescence in total (t), stromal (s), and intra-tumoral (i) tumor-infiltrating lymphocytes (TILs) of 49 metastasis samples (24 regional and 25 distant) from 22 cats with mammary carcinoma and regional and/or distant metastasis.

Results: We observed that the majority of the regional metastasis and of the distant metastasis expressed PD-1 and/or LAG-3 (71% and 72%, respectively), showing high levels of co-expression (94%). Interestingly, the LAG-3 expression occurred only in PD-1-positive metastasis and sTILs-PD-1/LAG-3+ were more frequent than iTILs- PD-1/ LAG-3+ (69%). Regarding the distant metastasis, although approximately 78% showed PD-1 or LAG-3 expression, most of the cases showed a low number of TILs.

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Conclusion: In sum, our results suggest that there is an inherent immune response within the tumor itself, reinforcing the limited effectiveness of immunotherapy in metastatic breast cancer. Furthermore, this study emphasizes the significance of evaluating metastatic sites to determine which cases are more likely to benefit from immunotherapy.

83. Metabolic-epigenetic landscape recoding in renal cell carcinoma: towards new therapeutic strategies

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Abstract

Introduction: Renal cell carcinomas (RCCs), particularly clear cell RCC (ccRCC) are the most lethal urological cancers due to disease dissemination and metastization. An in-depth understanding at the cellular and molecular level of ccRCC may provide further clues for the development of new therapeutic strategies that may reduce the above-mentioned cancer-related deaths. Recently, metabolite fluctuations were reported to dictate cancer cells' epigenetic plasticity, and their interactions were associated with cancer aggressiveness. Therefore, dissecting the relevance of the epigenetic-metabolic crosstalk for ccRCC aggressiveness and metastasis development might allow the discovery of new therapeutic targets to improve ccRCC patients' outcomes.

Material and Methods: A total of 200 ccRCC primary tumors and 25 normal kidney tissues derived from patients at IPO Porto were used for monocarboxylate transporters (MCTs), VHL, HIF-1α, sirtuins (SIRTs) imunoexpression analysis. (CES IPO:372/2017). The lactate effects on epigenetic enzymes, metabolic reprogramming, and cell phenotype were evaluated in normal kidney and ccRCC cell lines. Additionally, cells were exposed to sirtuin and MCT inhibitors, to investigate their impact in ccRCC cells, both in vitro, and in vivo (CAM model assay).

Results: MCT1, MCT4, HIF-1α expression was upregulated in ccRCC, whereas VHL was downregulated. Lactate was found to modulate histone deacetylases' expression, and specifically decreased SIRT1 and SIRT6 expression in ccRCC cell lines. SIRT1 and SIRT6 downregulation by lactate increased cell migration/invasion through the epithelial-mesenchymal transition and promoted a metabolic reprogramming switch.

Conclusion: MCTs overexpression, along with increased lactate levels, promoted ccRCC aggressiveness and induced malignant-like features in normal cells through SIRTs downregulation.

84. Zinc in the modulation of the DNA damage response: preventive, genotoxic, and cytotoxic roles in acute myeloid leukemia

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Abstract

DNA damage response (DDR) is key for genomic stability and zinc (Zn) is an important component of DDR. In acute myeloid leukemia (AML), Zn is commonly decreased, and DDR defects are observed. However, Zn's effects in DNA repair capacity of AML cells is not yet understood. We aimed to evaluate the role of Zn in DDR modulation in AML and address its therapeutic potential in combination with conventional therapy (cytarabine; AraC) and DDR inhibitors (olaparib; Ola).

AML cell lines (HEL, NB-4, K-562) and normal human lymphocytes (IMC) were used. Firstly, HEL and IMC were incubated in standard conditions (Std), Zn depletion (ZnD), and Zn supplementation (40μ M ZnSO4, ZnS) for 15 days, and then exposed to H2O2 and UV radiation. Chromosomal damage was evaluated by the micronucleus assay and DNA damage and repair by yH2AX expression. Then, the AML models were incubated for 72h with increasing AraC and Ola concentrations in monotherapy and combination with ZnSO4 (IC25). Cell density and viability were evaluated by trypan blue test, cell death (annexin V/7-AAD) and cell cycle (PI/RNAse staining) by flow cytometry. Expression of apoptosis (active caspase-3, cleaved PARP), DNA damage (yH2AX) and proliferation markers (BrdU) was assessed by flow cytometry. Data was analyzed considering a significance level of 95% (p<0.05).

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Zn increased the genotoxicity of H2O2 and UV radiation in AML cells and prevented damage accumulation in normal lymphocytes ($p<0.05$). Repair kinetics was compromised in ZnS AML cells ($p<0.05$). Zn reduced the IC50 of AraC about 6-19x and of Ola 3-9x in all AML cell lines ($p<0.05$). Combination schemes also increased apoptosis and induced G0/G1 arrest ($p<0.05$). Expression of apoptosis and DNA damage markers increased with Zn, while proliferation markers decreased ($p<0.01$). Zn supplementation improved the DDR in normal cells, and potentiated the cytotoxic, genotoxic and antiproliferative effects of therapeutic compounds in AML cells suggesting that Zn may lead to more efficient and less toxic therapeutic regimens.

85. Tumours with CDH1 Genomic Loss Have a Better Prognosis in Mice and Men

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Abstract

Introduction: Breast cancer is the most incidence cancer worldwide and the leading cause of cancer-associated deaths in females. Breast cancer's copy-number landscape and transcriptomic profile can define different IntClusters with distinguished molecular drivers and survival probabilities.

CDH1 is a tumour suppressor gene, with a proven role in gastric and breast carcinogenesis. Previous results indicate CDH1 intron 2 with regulatory features. Using in vivo experiments and in silico analysis of METABRIC cohort, we aim to explore the putative role of CDH1 intron 2 regulatory regions in breast cancer progression and prognosis.

Materials and Methods: We evaluated tumour burden in lungs from mice injected with CRIPSR-Cas9 clones harbouring distinct structural variants causing CDH1 impairment: one exonic and one intronic. Furthermore, we divided METABRIC patients into Homozygous deletions (HOMD), Heterozygous Deletion (HETD) and Amplification (AMP) - iCREs Study Groups - depending on the CDH1 intron 2 copy number and compared clinical-pathological and survival features.

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Results: Results demonstrate that cancer cells with CDH1 intron 2 impairment present lower tumour burden in mice lung's, suggesting a role for these regions in the metastasis process. Each iCREs Study Group presented distinguished clinical features, such as ER and HER2 status, tumour grade and NPI classifications. Evaluation of the tumour transcriptome of AMP and HOMD groups showed a closer profile with intronic edited clone, than with exonic edited clone. Kaplan-Meier survival analysis also suggests breast tumours with CDH1 intron loss displaying a better prognosis.

Conclusion: These findings support the hypothesis that CDH1 intronic regulatory elements play a role in lung colonization in mice and modulate breast cancer prognosis in humans.

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86. Escherichia coli "bioactive metabolites" modulate human prostate cells fate, oxidative stress and metabolic reprogramming

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Abstract

Introduction: The microbiome is a new dimension of cancer, which goes beyond tumor-promoting inflammation. The prostate cancer (PCa) bacteriome has been characterized; however, the importance of bacteria and their bioactive metabolites targeting different cancer hallmarks, such as metabolic reprogramming, remains unknown. Using a conditioned medium (CM) experimental approach, this work investigates the effect of Escherichia coli "bioactive metabolites" in PCa cells survival, oxidative stress, and metabolism.

Materials and Methods: E. coli-CM was produced by E. coli fermentation until the selected culture optical density was reached. Non-neoplastic prostate epithelial cells (PNT1A), androgen-sensitive (22Rv1), and castration-resistant (DU145) PCa cell lines were cultured with E. coli-CM for 24 and 48 h. Cell viability (MTT assay) and proliferation (Ki-67 immunocytochemistry) were evaluated. Caspase-3-like activity was used to assess apoptosis. Spectrophotometric analysis was used to determine protein carbonyl content, glucose and fatty acids consumption, lactate production, and glutathione peroxidase (GPx) and lactate dehydrogenase (LDH) activities.

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Results: Culture with E. coli-CM decreased the viability and proliferative capacity of PNT1A, 22Rv1, and DU145 cells, alongside increased caspase-3-like activity. Protein carbonyl content was increased in all cell lines, underpinned by the decreased GPx activity. Augmented fatty acid consumption was observed in PNT1A and DU145 cells cultured with E. coli-CM, followed by an increase in lipid content (PNT1A). Also, glucose consumption and lactate production, a tumor-like feature, were increased in DU145 cells despite decreased LDH activity.

Conclusions: E. coli "bioactive metabolites" seem to have a broad action over the PCa hallmarks deserving further investigation to disclose its protective or stimulating effects.

87. CITED2 as a Potential Therapeutic Target in Glioblastoma – Repurposing Pioglitazone

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Abstract

Glioblastoma is the most lethal type of brain cancer for which there are few therapeutic alternatives. The presence of cancer stem cells in glioblastoma may play a key role in the aggressiveness of these tumors. Glioblastoma stem cells (GSCs) appear to share self-renewal and fate mechanisms with embryonic and adult stem cells, in which CITED2 plays a relevant role. It was described that CITED2 participates in the maintenance of myeloid leukemia cells and treating these cells with Pioglitazone leads to the exhaustion of GSCs through the downregulation of CITED2. Since Pioglitazone can cross the blood-brain barrier, it is a potential candidate for drug repurposing, from diabetes to glioblastoma treatment. In this work we aimed to evaluate the effect of Pioglitazone on the viability of glioblastoma cells and the transcript levels of CITED2.

The effect of Pioglitazone on cell viability was evaluated through the colorimetric MTT assay in glioblastoma cell lines with different levels of CITED2, namely A172 and U118 (high CITED2) and LN229 (low CITED2) cells. For gene expression analyses, quantitative real-time PCR was performed using specific primers for CITED2 and CD44 (GSCs marker).

After 72 hours of treatment with 50 µM Pioglitazone, only a mild decrease of the viability of glioblastoma cells was observed. In response to Pioglitazone, CITED2 mRNA levels were increased by 2- to 3-fold in A172 and U118 cells. By contrast, CITED2 transcript levels were decreased in the LN229 cell line after treatment, compared to control vehicle (DMSO). While CD44 gene expression was undetectable in LN229, its transcript levels were increase in both A172 and U118 cells treated with Pioglitazone for 72 hours.

From these preliminary results we conclude that, despite the minor changes in cellular viability, CITED2 transcript levels are modulated in cells treated with Pioglitazone. Future perspectives include exploring the effects of the use of Pioglitazone in combination with other therapies, namely the alkylating agent Temozolomide.

88. Modulation of PARP1/2 and CHK1 as a novel treatment for acute myeloid leukemia - in vitro study

Authors and Affiliations

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Abstract

Genome integrity is threatened by various factors that can induce DNA damage. Thus, cells have developed mechanisms to preserve genome integrity, the DNA Damage Response (DDR). DDR includes sensor proteins (e.g., PARP1/2), mediators and signaling transducers, and effector molecules (e.g., CHK1/2). Genetic instability is characterized by increased DNA damage and alterations in DDR mechanisms, which may be implicated in the pathogenesis of acute myeloid leukemia (AML). This study aimed to evaluate DDR inhibition's therapeutic potential in AML through in vitro models.

Seven AML cells of different subtypes (HEL, HL-60, K-562, KG-1, LAMA-84, NB-4, and THP-1) were incubated in the absence or presence of increasing concentrations of two DDR inhibitors, CCT245737 (CHK1 inhibitor) and niraparib (PARP1/2 inhibitor). Cell density and viability were assessed using the trypan blue assay over 72 hours. Cell cycle analysis was performed using propidium iodide/RNase staining via flow cytometry, and cell death was determined using double staining with Annexin V/propidium iodide. The results were statistically analyzed with a significance level of 95%.

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CCT245737 and niraparib reduced cell proliferation and viability in a dose-, time-, and cell line-dependent manner. KG-1 cell line (IC50=56 μM at 24 hours) was the most sensitive to CHK1 inhibition, while NB-4 (IC50=53 μM at 24 hours) was the most sensitive to PARP1/2 inhibition. The cell line LAMA-84 was the most resistant to both inhibitors (IC50 at 24 hours of 189 μM and 106 μM for CCT245737 and niraparib, respectively). These inhibitors also led to an increase in the percentage of apoptotic cells. Additionally, a cytostatic effect was observed due to cell cycle arrest in the G0/G1 or G2/M phases.

Our results suggest that DDR inhibition could be a new potential therapeutic approach that could improve the treatment of AML patients. However, as cells lines exhibit different sensitivities to CCT245737 and niraparib, the efficacy of this approach may be dependent on the leukemia subtype.

89. interplay between pancreatic tumors and immune selection at the thymus

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is often characterized as a "cold tumor", with limited immune infiltration and a low response to immunotherapy. PDAC's cold tumor status is due to a complex interplay of factors within the tumor microenvironment. It typically exhibits a dense stroma and immunosuppressive milieu, which hinders the infiltration of immune cells. Additionally, PDAC cells employ various immune evasion mechanisms, making it challenging for the immune system to mount an effective response against cancer.

Emerging evidence underscores the pivotal role of exosomes in cancer. Exosomes carry bioactive cargo capable of reprogramming neighboring and distant cells. Among the multifarious constituents of cancer exosomes (cExos), major histocompatibility complex class I and II molecules (MHC-I/II) are included, albeit their precise contribution remains enigmatic. In here, we proposed to investigate the role of cExos in the modulation of the immune response.

Utilizing unique genetically engineered mouse models (GEMMs) that spontaneously develop PDAC, wherein cancer cells produce fluorescently tagged cExos, we unveil a pancreas-thymus communication axis in PDAC and also in healthy conditions. Interestingly, GEMMs that have impaired cExos secretion display an expanded thymic medulla-to-cortex ratio. This observation leads us to postulate that cExos could play a pivotal role in modulating negative selection processes within the thymus by presenting tumor-specific antigens to thymocytes, which subsequently perceive these antigens as self, leading to their elimination. This mechanism could effectively thwart the activation of T-cells capable of recognizing and mounting an immune response against the tumor.

We propose to investigate a novel facet of cExos in shaping the immune response within PDAC, offering a fresh perspective for the development of therapeutic strategies aimed at dismantling the immune-suppressive milieu that characterizes these tumors.

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90. Glyco-Immune Checkpoint in Colorectal Cancer: the role of ST6Gal1 glycosyltransferase

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Abstract

Introduction: Current therapeutic approaches have been successfully employing immune checkpoint inhibitors to boost native anti-tumor responses and improve the clinical outcome of cancer patients. Despite the favorable results, a fraction of cancer patients still does not benefit from immunotherapy urging the search for novel tumor neoantigens and therapeutic targets, as well as a better understanding of the mechanisms behind tumor immune evasion. Hypersialylation is an immunosuppressive glycosylation pattern frequently found in malignant cells, hindering anti-tumor immunity. This study aims to investigate the role of α 2,6-sialylation in the crosstalk between tumor and immune cells.

Materials and Methods: We used glycoengineered colorectal cancer cell models with differential expression of the sialyltransferase ST6Gal1 and CRISPR/Cas9-mediated abrogation of crucial sialic acid receptors on immune cells to perform co-cultures between tumor cells and macrophages.

Results: Here, we show that the tumor-associated sialyltransferase ST6Gal1 generates sialylated ligands of an inhibitory receptor from the family of sialic acid-binding lectins, and that $\alpha 2,6$-sialylated tumor cells are able to evade macrophage-mediated phagocytosis through a mechanism dependent on a specific sialic acid-binding lectin. Moreover, this glyco-immune axis was found to be clinically relevant for colorectal cancer, particularly those harboring tumors with a microsatellite stable phenotype.

Conclusions: These results disclose the role of ST6Gal1 in cancer immunosurveillance and as a suppressor of tumor cell clearance, providing new clinical targets for cancer treatment.

91. Fibroblasts, cancer stem-like cells, and KRAS-inhibited tolerant cells: The three resisters united to preclude therapy response

Authors and Affiliations

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Abstract

KRAS-targeted therapies are a promising approach for treating a subset of cancer patients with KRAS mutations, but response rates are lower than expected and many patients quickly develop resistance to therapy.

Emerging evidence suggests that cancer stem cells (CSCs) and cancer-associated fibroblasts (CAFs) may have a role in conferring resistance to therapy. This led us to explore whether CAF-derived factors drive a stem-like phenotype, contributing to KRAS-targeted therapy resistance.

CRC cell lines HCT15, HCT116 and SW480 were cultured either in recommended media or in conditioned media (CM) from normal colon fibroblasts cell line (CCD-18co) either activated with TGF-β (CAFs) or not. Expression of membrane stem cell markers (SCM) was analyzed by flow cytometry (FC). Stem cell potential was evaluated by in vitro colonosphere formation assay. RNAseq analysis was performed in KRAS silenced HCT116 colonospheres treated with either control media or CM of CAFs. NOTCH inhibition was done with a gamma-secretase inhibitor.

Basal SCM expression by FC in KRAS-mutated CRC cell lines revealed distinct stem marker signatures. KRAS silencing increased CD24 expression and decreased integrins α6 and β4 across cell lines, indicating reduced stemness, also supported by decreased colonosphere-forming efficiency (CFE). Culturing these cells in CAF-derived conditioned media resulted in minimal changes in stem marker expression. Surprisingly, the media restored CFE, overcoming the inhibitory effects of KRAS silencing. RNAseq analysis in KRAS-silenced cells treated with control vs. CAFs-conditioned media suggested the involvement of NOTCH signaling in CFE recovery supported by the decreased CFE upon NOTCH inhibition.

We show a novel resistance mechanism to KRAS inhibition involving the activation of CSCs mediated by CAF-derived factors, potentially through the NOTCH signaling pathway. This KRAS-independent mechanism may explain the limited efficacy of KRAS-targeted therapies and offer new options to improve CRC treatment.

92. Functional assays reveal a role for CITED2 in the stemness properties of glioblastoma

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Abstract

Glioblastoma (GBM) is a type IV glioma and the most lethal primary brain tumor. This is mainly due to the current few treatment options. Accumulating evidence shows that these tumors are composed of a heterogeneous population of cells that originates from a smaller subset known as Glioblastoma Stem Cells (GSCs). GSCs were shown to present relative quiescence and to be resistant to standard chemotherapy and radiation, which target mainly bulk cells, leading to relapse. Therefore, therapeutic approaches targeting these cells may prevent relapse but the molecular mechanisms regulating their maintenance are poorly understood. CITED2 is a co-transcriptional regulator and a key pluripotency factor in embryonic and adult stem cells. This protein was implicated in several types of cancer either with pro-tumorigenic or anti-tumorigenic roles. By analyzing the publicly available datasets Genotype-Tissue Expression (GTEx) and The Cancer Genome Atlas (TCGA), including the lower grade glioma (LGG) and GBM cohorts, we found that CITED2 is more expressed in GBM when compared to LGG and that high CITED2 expression in gliomas leads to an adverse prognosis. Nevertheless, a role for CITED2 in glioblastoma was not previously reported. To determine if CITED2 impacts the self-renewal capacity of GSC and other biologic properties, we generated CITED2 knockdown and overexpression GBM stable cell lines. Then, we evaluated the impact of CITED2 modulation on proliferation, cell cycle progression, viability, differentiation, and invasive potential of GSC, by performing a set of in vitro assays. Although the knockdown was challenging, CITED2 overexpression revealed its impact on the self-renewal properties of the LN229 cell line. Therefore, modulating CITED2 expression in glioblastoma may be an effective approach to interfere with the stemness properties of GSCs and potentially lead to a better prognosis.

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93. Genomic insights unveiled: A comprehensive WGS pipeline for CNV and SNV discovery

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Abstract

Introduction: Exome analyses fail to detect single nucleotide variants (SNVs) or structural variants (SV) occurring outside the coding sequence. Genome-wide analysis emerges as a solution to understand genome variation, and Whole Genome Sequencing (WGS) the preferred method for that purpose. We aimed at developing a WGS analysis workflow using a combination of multiple variant callers for SNV, CNV and SV calling.

Methods: We used a gold standard sample from the Genome in a Bottle project (GIAB) to access the performance of a novel pipeline encompassing alignment (BWA mem); post-processing (GATK tools); CNV (integration of LUMPY, Delly and GRIDSS caller outputs) or SNV (integration of HaplotypeCaller-GATK (HC) and DeepVariant (DV) outputs) calling; and Merging of multiple called variants (overlap analysis of SNV, CNV and SV calls). We calculated recall, precision and F1 scores by comparing our outputs with those from GIAB for SNVs. As no gold standard is available in GIAB for CNVs, we compared our CNV outputs with a GIAB pool of high confidence and with variant calls from Manta, to improve our pipeline.

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Results: We called 4.309.554 SNVs with DV and 4.578.886 with HC, and 4.109.099 were common. The performance of DV alone (F1 score=0.9819, 3.802.474 true positives) was better than SNV calling with HC alone (F1 score=0.9522, 3.759.774 true positives), but worse than the combination of DV and HC outputs (F1 score=0.9841, 3.836.476 true positives variants). Our CNV calling pipeline called a higher number of variants than the, as well as a set of inversions confirmed by visualization in samplot and supported paired-end and/or split-reads, that were not called by GIAB or Manta.

Conclusion: Our WGS-pipeline shows high performance and improves the likelihood of finding true positive germline SNVs, captures high confidence CNVs and uniquely calls inversions.

94. Deciphering the contribution of pancreatic stellate cell secretome in pancreatic cancer drug response

Authors and Affiliations

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Abstract

Pancreatic stellate cells (PSCs) and cancer-associated fibroblasts (CAFs) play an important role in desmoplasia, a primary contributor to treatment resistance in pancreatic ductal adenocarcinoma (PDAC). Several pro-tumorigenic factors have been identified within the secretome of PSCs and CAFs, either in the form of soluble proteins or associated with Extracellular Vesicles (EVs).

The aim of this study is to identify mediators of PDAC drug resistance in the secretome of PSCs, namely in the EVs shed by these cells.

To achieve this, we selected the human pancreatic stellate cell line HPaSteC, alongside the human dermal fibroblast cell line HFF-1 (used as a negative control). The effect of the secretome of both cell lines is being evaluated on cell viability and drug response (to gemcitabine and paclitaxel) in spheroids of the Panc-1 and AsPC-1 PDAC cell lines. Moreover, we are conducting an analysis of spheroids' morphology (area, diameter, circularity and compactness) and evaluating the expression of epithelial-mesenchymal transition (EMT) proteins by Western Blot (WB). To investigate the contribution of PSCs-derived EVs on PDAC drug response, EVs secreted by HPaSteC and HFF-1 cell lines are being isolated by differential ultracentrifugation and characterized by transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA) and by WB for EV biomarkers, according to the Minimal information for studies of extracellular vesicles (MISEV) 2018 guidelines.

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The proteome of the EVs is being analyzed by liquid chromatography-mass spectrometry and will be validated by Western Blot. Future work will assess the direct contribution of PSCs-derived EVs on the viability and drug response of the same PDAC 3D cell model.

With this work, we hope to identify key proteins involved in PSCs-mediated drug response in PDAC, with potential as therapeutic targets or as biomarkers.

95. Adipocytes modulate tumour microenvironment of inflamed Lymphomas

Authors and Affiliations

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Abstract

Introduction: The increase of 5 kg/m2 in body mass index has been associated with a significant rise in Hodgkin's lymphoma (HL) risk. The HL has a unique tumour microenvironment (TME) crammed with infiltrating immune populations. Adipocytes have been implicated as culprits in obesity-cancer association. We sought to unravel adipocyte's modulatory role in the crosstalk between cancer-immune cells.

Materials and Methods: We developed a tetra-culture model with adipocytes, macrophages, T cells and L428 cells. The human adipocytes were collected from obese patients submitted to bariatric surgery. Macrophages and T cells were isolated from healthy blood doors. After 72h of culture, we characterize the condition media using a Bio-Plex multiplex cytokine array, performed the analysis of cell surface markers by cytometry. We perform gene expression characterization of adipocytes. The validation of data was performed in patient derived samples using perilymphodal adipocytes (PAT) and immune cells.

Results: Adipocytes induced a pro-inflammatory macrophage, and T cell activation in tetraculture. Subsequently, the soluble factors corroborated cytometry findings. Moreover, when in tetraculture adipocytes, presented an impaired mitochondrial function with decreased PPARG gene expression and changes in immune tolerance through increased expression of PD-L1. Finally, in an autologous system, we were able to validate the tetraculture system with patient-derived samples, namely, the PAT induced the activation of T cells, and pro-inflammatory macrophages.

Conclusions: Taken together, this tetra-culture model demonstrates an immunomodulatory role for adipocytes at the HL TME, reflecting the importance of adipose tissue in HL mechanisms.

96. P-selectin glycoprotein ligand-1 (PSGL-1) inactivation in T cells leads to stronger T cell activation in response to TCR stimulation

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Abstract

Introduction: The P-selectin glycoprotein ligand-1 (PSGL-1), encoded by the SELPLG gene, is a transmembrane protein expressed in hematopoietic cells. It plays an important role in tethering, rolling and extravasation of immune cells from blood vessels. It functions as a signal transduction receptor and modulates responses of PSGL-1-expressing T cells to specific stimuli. It was reported that PSGL-1 can regulate the immune function of T cells, by regulating negatively antitumor T cell responses, promoting T cell exhaustion and allowing tumor growth. We hypothesized that PSGL-1 acts as an immune checkpoint protein by interfering with T cell activation.

Materials and methods: To evaluate ERK and ZAP-70 phosphorylation upon CD3/TCR activation, we performed Western blot and flow cytometry analysis. This was performed in stimulated (PMA plus ionomycin, anti-CD3 or CD3/CD28 beads) and unstimulated parental, mock and SELPLG KO Jurkat cells. T cell activation markers CD69, PD-1 and CD25 were assessed by flow cytometry in the mock and SELPLG KO cell lines stimulated with anti-CD3 and unstimulated. To evaluate levels of p-ERK upon TCR/CD3 stimulation and treatment with a PL1 antibody in healthy donor T cells, we performed flow cytometry analysis.

Results: Firstly, to explore the TCR/CD3 signaling pathway activation, we analyzed the phosphorylation of ERK. SELPLG KO did not affect p-ERK and p-ZAP-70 levels in Jurkat cells upon anti-CD3/CD28 beads and PMA plus ionomycin stimulations. The expression of T cell activation markers was similar between mock and SELPLG KO cells in the 2, 4 and 6 h timepoints upon TCR/CD3 stimulation. However, at 16 and 24 h, CD69 and CD25 expression levels were higher in the SELPLG KO cell line. Lastly, healthy donor T cells treated with PL1 showed a cumulative effect in p-ERK expression, upon anti-CD3 stimulation.

Conclusions: The absence of PSGL-1 does not seem to affect TCR/CD3 signaling. PSGL-1 inactivation increased the percentage of activated T cells, and the target of PSGL-1 can increase the activation status of T cells.

97. Tumor-hematopoietic communication in colorectal cancer escape from immunosurveillance

Authors and Affiliations

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Abstract

Introduction: The immunoediting comprises an initial elimination phase targeting nascent tumor cells, followed by an equilibrium phase wherein the immune response and tumor cells enter a state of homeostasis, temporarily constraining tumor expansion. Tumors eventually escape immunosurveillance, leading to the development of detectable benign tumors and escalating the risk of malignant progression. Our aim was to identify the genetic, cellular, and molecular drivers of escape to immunosurveillance.

Results: Using genetically engineered mouse models of colorectal carcinogenesis, we observed that oncogenic Kras accelerated tumor growth in a Msh2 null background compared with single mutants. The Msh2/Kras mice displayed multiple tumors throughout the intestine, splenomegaly, and signs of anemia, alongside histologic and cytologic abnormalities within both the bone marrow and thymus. A detailed flow cytometry analysis of the spleens of Msh2/Kras tumor-bearing mice revealed alterations in distinct immune cell populations compared with age-matched wild-type and tumor-free single mutant mice. Additionally, the number of tumors positively correlated with spleen size and negatively correlated with the percentage of CD45+ cells in the spleen. Our current focus is understanding the mediators of crosstalk between tumors and hematopoietic organs, which we evaluated using a multiplex cytokine array. Our team is also continuing to investigate the impact of these changes on the tumor immune microenvironment.

Conclusion: Our study highlights a novel immunosurveillance escape mechanism involved in oncogenic KRAS-driven intestinal tumorigenesis through long-distance communication with hematopoietic organs. Understanding this mechanism is essential for the development of effective treatments that enhance the immune response against early-stage tumors, thereby preventing their escape, and ultimately curbing their progression towards malignancy.

98. A Bispecific antibody to block iron-endocytosis-driven metastasis

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Abstract

Cancer remains the second leading cause of death and morbidity worldwide, with 10 million estimated deaths in 2020. Metastasis is the main contributor to cancer mortality and morbidity. Metastatic breast cancer (MBC), accounts for the vast majority of the 0.6 million deaths from breast cancer each year globally Despite the advances in chemotherapy, the risks associated with current therapies still carry severe side effects and offer limited benefit to the patient. Thus, there is a critical need for innovative drugs capable of modulating new cancer-relevant targets. Evidence suggests that targeted therapies may provide an original means of selectively modulating diseased breast tissues. We proposed to validate and develop an unprecedented approach to tackle MBC, by targeting the overexpressed CD44 Hyaluronic Acid (HA) and the transferrin receptor 1 (TfR1)-transferrin pathways, with a bispecific antibody (bsAb). Since metastasis relies on the epithelial-mesenchymal plasticity of cancer cells, and this plasticity requires a change in gene expression, blocking iron endocytosis will block histone demethylation required to unlock the expression of mesenchymal and metastatic genes.

We have already screened a subset of 7 promising antibodies that bind to TfR1 and CD44 and validated the blocking effect of each individual ab selected on the uptake of labeled transferrin and Hyal using flow cytometry and confocal microscopy. Intracellular iron contents were assessed using RhoNox-M, a turn-on fluorescent probe for the selective detection of iron(II). TfR1 and CD44 expression levels were assessed in different MBC cell lines. Several antibodies showed high binding affinity towards their targets as well as a reduction of iron uptake in MDA-MB-468 cell line.

We strongly believe that through simultaneous disruption of iron signaling and consequently blocking epigenetic changes using a bsAb built based on the best parental molecules selected from our screens, we will control the expression of metastasis genes and selective targeting and killing of MBC cells will be achieved.

99. T-cell receptor induction of CCR7 chemokine receptor promotes leukemic T cell dissemination

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Abstract

T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL) involves mainly the bone marrow, blood, and lymphoid organs, but can disseminate to other organs, aggravating disease. It was previously reported by us and others that absence of T-cell receptor (TCR) expression in T-ALL/LBL mouse models led to reduced dissemination of leukemic cells from the thymus to peripheral lymphoid organs, most notably lymph nodes. We aimed to understand which molecules could be involved in this TCR-dependent property. Infused ETV6::JAK2 transgenic (EJ-Tg) mouse leukemic T cells lacking TCR expression (i.e., EJ-Tg;Rag2-/-) colonized much less efficiently the lymph nodes and spleens of recipient mice than EJ-Tg leukemic cells expressing endogenous or transgenic HY-TCR. Interestingly EJ-Tg;Rag2-/-leukemic cells expressed reduced levels of the Ccr7 chemokine receptor, a T-cell migration mediator involved in both thymic egress and peripheral lymphoid organ homing. By stimulating human T-ALL cell lines with CD3 antibody or the PMA phorbol ester, we confirmed that CCR7 expression was induced by TCR signaling. To study the role of CCR7 in vivo, we bred EJ-Tg mice with Ccr7 KO mice. Similarly to EJ-Tg mice lacking TCR (i.e. Tcr α -/- or Rag2-/-), EJ-Tg;Ccr7-/- mice presented significantly larger thymic lymphomas and reduced splenic and lymph nodal involvement than Ccr7-sufficient littermates. By breeding EJ-Tg mice with OT-I TCR transgene and Ccr7 KO, we observed that the absence of CCR7 in OT-I;EJ-Tg mice resulted in lower lymph node and spleen dissemination of leukemic cells. To verify whether leukemic cells expressing Ccr7 was involved in homing to lymphoid organs, Ccr7-expressing EJ-Tg leukemic cells were infused in mice KO for the Nf κ b2 transcription factor, which express reduced levels of Ccl19 and Ccl21, the Ccr7 ligands, in the lymph nodes, or control littermates. Infused leukemic cells colonized less efficiently the lymph nodes of Nf κ b2-deficient mice, with no differences in the spleen and liver. We conclude that TCR signaling is associated with expression of proteins associated with leukemic dissemination to specific niches and that CCR7 is a potential mediator of that property.

100.   Development of novel CAR-Ts directed to tumor-associated glycoepitopes

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Abstract

Introduction: CAR-T cell technology shows promise in treating hematologic malignancies, but solid tumors pose challenges due to the absence of suitable targets. Despite glycans' role in tumor progression, they remain underexplored as CAR-T targets. Truncated O-glycans, highly expressed in epithelial tumors and rarely detected in healthy tissues, constitute promising candidates. Here, we generated and characterized novel monoclonal antibodies (mAbs) targeting short O-glycans and subsequently developed new CAR-Ts.

Materials and Methods: New mAbs targeting short O-glycans were produced by hybridoma technology. ELISA, glycan array, western blotting, flow cytometry, immunofluorescence and immunohistochemistry were performed to characterize the new antibodies.

The sequences of the scFv were extracted to create second-generation molecules that were subsequently expressed in T cells.

The functionality of the CAR-Ts was assessed through in vitro assays by co-culturing the manipulated T cells with target- and target+ cancer cells. In vivo tests on murine xenograft models were also carried out to evaluate the CAR-Ts' ability to control tumor growth.

Results: AM51.1 and AM52.1 clones demonstrated high specificity, as confirmed by ELISA and glycan array. Western blotting, flow cytometry and immunofluorescence showed binding to the glycan of interest in tumor cells. Immunohistochemistry validated the mAbs' high specificity for the target present in human gastric, colorectal, pancreatic, and ovarian cancer tissues, with no recognition of healthy tissues.

Second-generation CARs were created from the novel mAbs, and flow cytometry confirmed their expression on T cell surface.

In vitro and in vivo tests revealed potent functionality of the new CAR-Ts, effectively killing target+ cells and controlling tumor growth.

Conclusions: In conclusion, our findings demonstrate the potential of the novel glycan-specific CAR-Ts as an effective therapeutic option against a large proportion of carcinomas.

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